COMPLEMENTARY ASSEMBLY PROCESSES ACROSS BIODIVERSITY GRADIENTS

Dissertation

zur

Erlangung der naturwissenschaftlichen Doktorwürde

(Dr. sc. nat.)

vorgelegt der

Mathematisch-naturwissenschaftlichen Fakultät

der

Universität Zürich

von

Alexander Jon Fergus

aus

Neuseeland

Promotionskomitee

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For Ma, who would have wanted to have seen this thing finished.

Ma and Pa visiting the Jena Experiment in 2008.

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Summary

Community assembly processes reveal the direction and strength of species interactions. Plant community structure is determined by these interactions and understanding community structure permits a mechanistic insight into the forces driving ecosystem functioning. Community assembly arises from three hierarchical processes: the regional species pool determines which species have viable local populations and can potentially colonise a given site within a region; the local abiotic conditions at a given site select for species from the regional pool with the appropriate range of traits required to grow and reproduce there; biotic interactions – including competition, mutualism and exploitation – govern local community composition. In this thesis I have examined how biotic interactions shape community assembly across biodiversity gradients, considering gradients of species richness, functional group richness, or metrics of evolutionary distance and diversity.

The number of species in a community can alter the dynamics of interspecific interactions, and can influence important properties of communities such as their resistance to alien plant invasion or the provisioning of ecosystem functions and services. In reviewing biodiversity–ecosystem functioning relationships we have concluded that biodiversity has significant positive impacts on community production of biomass and associated processes (chapter 1). We have experimentally demonstrated that many ecosystem functions respond to plant species richness, particularly processes involving carbon cycling, and we have also found support for positive bottom-up effects of plant species richness on higher trophic levels (chapter 2).

Plant communities are not solely comprised of vascular plant species; consequently we followed the assembly of bryophytes along a vascular plant species richness gradient (chapter 3). Bryophyte richness responded negatively to this gradient and bryophyte community composition varied distinctly along it. By implication, advocating increased richness of one taxonomic group over another potentially impacts collective community diversity. We also considered how other taxonomic groups below ground structure plant communities, which challenged prevailing niche-based dogma for explanations of biodiversity-ecosystem functioning relationships. We demonstrated that negative plant–soil feedbacks depress site re-occupation success by a species belonging to the same functional group, effectively promoting species coexistence and

diversity (chapter 4). Effects were driven by soil pathogens and were compounded by interspecific competition. This prompted us to theoretically explore how natural enemy regulation influences establishment. Models were constructed whereby soil pathogens reduced the competitive ability of resident species in sites formerly occupied by the same species (chapter 6). The ability to immigrate and establish was dependent on the competitive ability of the incoming species, regardless of pathogen regulation, as the abundance of the incoming species is initially low. The success, or population density, of an incoming species was however dependent on pathogen-regulation. With increasing community diversity resident species are less likely to occupy a site formerly occupied by the same species, reducing pathogen regulation, and removing any depressed impact on the competitive ability of the resident community. The upshot is that in pathogen regulated communities increasing community diversity increases community resistance to incoming species, or if we consider a scenario involving alien species, more diverse communities have greater invasion resistance. We tested these theoretical results under field conditions in established communities, using two metrics of establishment success to reveal shifts between life history stages (chapter 5). Immigrant seedling abundance was always higher in communities containing the same functional group, probably due to abiotic facilitation or shared mutualists. This pattern was opposite or indistinct after immigrants had established, therefore biotic interactions can sequentially drive assembly in different directions.

In a more complex system, we have demonstrated using seed addition experiments that immigrant species complement communities with initially low richness, leading to convergence of species richness, functional group richness and evenness across different communities (chapter 7). Similarly, in the same experimental platform, we showed that spontaneous colonisation by immigrants – after we ceased to enforce a richness gradient – has less impact on the abundance and stability of communities with higher initial species richness (chapter 8).

There is an evolutionary backdrop to these feedbacks and patterns of reassembly. Across the initial sown species richness gradient the abundance distributions of species in communities became overdispersed with time (chapter 9). This phylogenetic overdispersion was evident as dominant species in a community

being more distantly related than expected based on the performance of individual species in monoculture, suggesting that species interactions limit the similarity of at least dominant species. If lineages retain inherited environmental preferences, but also explore novel environments, then we could expect biotic interactions to drive co-existence of related species in different directions. We removed dispersal limitation and added the full complement of species in our experimental species pool to all plots (e.g. across the initial sown species richness gradient). As communities reassembled they converged on similar levels of phylogenetic diversity (chapter 10). The correlation between species co-occurrence and phylogenetic distance developed with time from initial phylogenetic clustering (species are more closely related than expected at random) to phylogenetic overdispersion. Broken down, the pattern of co-occurrence and phylogenetic distance revealed increased clumping of close and distantly related species. Examining these patterns separately for different lineages, we identified contrasting coexistence patterns, suggesting different levels of phylogenetic dispersion could drive interactions within a lineage. From large observational databases we found that certain types of plant communities assembled from fewer phylogenetic lineages (in plots without alien plant species) were increasingly vulnerable to invading alien species, the addition of which increased dispersion of most traits (chapter 11). Communities with more alien species also had higher functional redundancy, which generally increased with total species richness, demonstrating increased trait state similarity (chapter 12). Coexistence of species exhibiting similar trait states suggests shared, as opposed to partitioned, resource use, indicating that niche-based mechanisms alone cannot explain coexistence, and reinforcing the role that natural enemies (e.g. pathogens) or mutualisms have in structuring plant communities.

A caveat to this research is the limitation of experimental design; we witnessed the feedback from biodiversity to ecosystem function, but in isolation of a number of natural processes. However, in doing so, we cut to the heart of species interactions and advance our understanding of how they drive ecological processes. I have demonstrated ways in which community assembly processes can be driven by species interactions. These interactions are heavily contingent on evolutionary history, which dictates how species richness influences biotic interactions. I have shown that

community assembly is a complementary process, but that it is not limited to resource based explanations. Niche complementarity in tandem with our growing understanding of pathogen regulation has advanced our understanding of the critical biological drivers of the assembly processes presented in this thesis.

The examination of assembly processes across biodiversity gradients permits further insight into the impacts that species loss will have on ecosystem functioning. Changes in ecosystem functioning go beyond variation in community productivity, to core biogeochemical processes and then reverberate across trophic levels. Increasing species richness likewise reinforces functional and compositional richness that stabilizes both ecosystem processes and the green platform that supports organisms at higher trophic levels. The growing evidence for the significance of phylogenetic metrics further suggests how species richness incorporates highly valuable information concerning heritable variation that we cannot yet measure, such as pathogen association. Beyond contributing to the fundamental understanding of how communities and ecosystems are structured and operate, these results have immediate and profound implications for ecosystem management, restoration and sustainable agriculture. Under threat of species loss from changing climatic variables, intensifying land-use and expanding alien invasive species, this research specifically reinvigorates one key principle – that biodiversity is critically important.

Zusammenfassung

Prozesse der Gemeinschaftsbildung veranschaulichen die Art, Ausprägung und Intensität von Artinteraktionen. Die Struktur von Pflanzengemeinschaften wird durch diese Interaktionen bestimmt und das Verständnis für die Gemeinschaftsstruktur erlaubt einen mechanistischen Einblick in die treibenden Kräfte der Ökosystemfunktionen. Die Bildung von Artgemeinschaften erfolgt aufgrund dreier sukzessiver Prozesse: der regionale Artenpool bestimmt welche Arten überlebensfähige lokale Populationen aufweisen und potentiell in der Lage sind ein bestimmtes Gebiet innerhalb einer Region zu besiedeln. Innerhalb dieses bestimmten Gebietes selektieren die lokalen abiotischen Bedingungen die Arten, die das, für das Wachstum und die Reproduktion notwendige Merkmalspektrum aufweisen. Biotische Faktoren und Interaktionen, wie Konkurrenz, Mutualismus und Ausbeutung, regulieren wiederum die lokale Zusammensetzung der Gemeinschaft. In der vorliegenden Arbeit habe ich untersucht wie biotische Interaktionen entlang von Biodiversitätsgradienten die Bildung von Artgemeinschaften beeinflussen; und dieses unter Einbeziehung der Artenvielfalt, der funktionellen Vielfalt von Artengruppen oder Kenngrössen evolutionärer Distanz und Diversität.

Die Artenzahl in einer Gemeinschaft kann die Dynamik von interspezifischen Interaktionen verändern. Zudem kann sie wichtige Eigenschaften der Gemeinschaft, wie zum Beispiel Resistenz gegenüber Invasionen fremder Pflanzenarten oder die Bereitstellung und Gewährleistung von Ökosystemfunktionen und Ökosystem-Service-Leistungen beeinflussen. Anhand der Bewertung von Untersuchungen zur Beziehung zwischen Biodiversität und Ökosystemfunktion folgerten wir, dass Biodiversität einen signifikanten positiven Einfluss auf die Produktion von Biomasse und der damit verbundenen Prozesse hat (Kapitel 1). In einem Experiment haben wir gezeigt, dass viele Ökosystemfunktionen auf die Vielfalt von Pflanzenarten reagieren, insbesondere Prozesse des Kohlenstoffzyklus. Ausserdem haben wir Hinweise für positive „Bottom-up“ Effekte von Pflanzenartenvielfalt auf höhere trophische Ebenen gefunden (Kapitel 2). Pflanzengemeinschaften bestehen nicht ausschliesslich aus vaskulären Pflanzenarten; somit verfolgten wir die Anordnung und Zusammenfügung von Bryophyten entlang eines Gradienten von vaskulärer Pflanzenartenvielfalt (Kapitel 3). Die Bryophytenvielfalt nahm entlang dieses Gradienten ab und die Zusammensetzung der Bryophytengemeinschaft variierte deutlich. Folglich beeinflusst die Förderung von

erhöhter Vielfalt einer taxonomischen Gruppe gegenüber einer anderen potentiell die gesamte Diversität einer Gemeinschaft. Wir berücksichtigten auch wie andere taxonomische Gruppen im Boden Pflanzengemeinschaften beeinflussen, welches das allgemein geltende nischenbasierte Dogma zur Erklärung von Beziehungen zwischen Biodiversität und Ökosystemfunktionen infrage stellte. Wir demonstrierten dass negative Pflanzen-Boden-Rückkoppelungen den Erfolg der Wiederbesiedlung eines Gebietes durch eine Art, welche zugehörig zur gleichen funktionellen Artengruppe ist, vermindern und so gewissermassen die Koexistenz und Diversität von Arten begünstigen (Kapitel 4). Die Auswirkungen wurden durch Bodenpathogene gesteuert und durch interspezifische Konkurrenz verstärkt. Dies hat uns dazu veranlasst, theoretisch zu untersuchen wie die natürliche Regulierung von Feinden die Besiedlung und Etablierung beeinflusst. Modelle wurden konstruiert wonach Bodenpathogene die Konkurrenzfähigkeit ansässiger Arten in Gebieten, ehemals besiedelt von der gleichen Art, reduzierten (Kapitel 6). Ungeachtet der Regulierung durch Pathogene, war die Fähigkeit zur Einwanderung und Etablierung abhängig von der Konkurrenzfähigkeit der jeweiligen Art, da die Abundanz der einwandernden Art anfangs gering ist. Allerdings war der Erfolg oder die Populationsdichte einer einwandernden Art abhängig von der Regulierung durch Pathogene. Mit steigender Diversität der Gemeinschaft sinkt die Wahrscheinlichkeit ansässiger Arten ein Gebiet zu besiedeln dass ehemals von der gleichen Art besiedelt war, welches wiederum die Regulierung durch Bodenpathogen reduziert und jeglichen unterdrückenden Einfluss auf die Konkurrenzfähigkeit der ansässigen Gemeinschaft aufhebt. Das Fazit ist, dass in durch Pathogene regulierten Gemeinschaften steigende Diversität die Resistenz gegenüber einwandernden Arten stärkt, oder im Falle eines Szenarios unter der Einbeziehung gebietsfremder Arten die Resistenz gegenüber der Invasion der selbigen. Wir untersuchten diese theoretischen Annahmen und Ergebnisse in etablierten Gemeinschaften und unter natürlichen Bedingungen im Freiland. Um Verschiebungen zwischen den Abschnitten des Lebenszyklus aufzuzeigen, verwendeten wir zwei Kenngrössen für den Etablierungs- bzw. Kolonisationserfolg (Kapitel 5). Die Keimlingsabundanz der eingewanderten Arten in Gemeinschaften, welche die gleiche funktionelle Artengruppe enthielten, war immer grösser, wahrscheinlich aufgrund von abiotischer Begünstigung oder aufgrund

gemeinsamer Mutualisten. Dieses Muster war entgegengesetzt oder unbestimmt nachdem sich einwandernde Arten etabliert hatten. Demzufolge können biotische Faktoren fortlaufend die Zusammensetzung in unterschiedlicher Weise beeinflussen und steuern.

Mittels Einsaat-Experimenten haben wir gezeigt, dass in einem komplexeren System eingewanderte Arten Gemeinschaften mir einer geringen Anfangsvielfalt ergänzen, was zur Konvergenz von Artenvielfalt, Vielfalt funktioneller Artengruppen und Äquität (Gleichheit) quer durch verschiedene Gemeinschaften führt (Kapitel 7). In demselben experimentellen Aufbau zeigten wir in ähnlicher Weise dass spontane Kolonisation durch Einwanderer einen geringeren Einfluss auf die Abundanz und die Stabilität von Gemeinschaften mit einer höheren Anfangsvielfalt hat – nachdem wir die Erzwingung und Verstärkung eines Artenvielfalt-Gradienten beendet hatten (Kapitel 8).

Es besteht ein evolutionärer Hintergrund zu diesen Rückkoppelungen und Mustern des wiederholten Zusammenschlusses. Mit der Zeit zeigten die Abundanz-Verteilungen der Arten in Gemeinschaften eine Überdispersion entlang des anfangs gesäten Artenvielfaltsgradienten (Kapitel 9). Diese phylogenetische Überdispersion war erwiesen da dominante Arten in einer Gemeinschaft entfernter in Beziehung standen als man basierend auf der Leistungsfähigkeit einzelner Arten in Monokultur erwarten würde. Dies suggeriert dass Artinterkationen die Ähnlichkeit von wenigstens dominanten Arten begrenzt. Falls die Abstammungslinien vererbte Umwelt-Präferenzen beibehalten – aber auch neuartige Umgebungen erkunden – dann könnten wir erwarten dass biotische Interaktionen die Koexistenz von ähnlichen Arten in unterschiedlicher Weise beeinflussen und steuern. Wir entfernten Ausbreitungslimitierung und fügten die volle Anzahl an Arten aus unserem experimentellen Artenpool allen Plots hinzu (z.B. entlang des anfangs gesäten Artenvielfaltsgradienten). Indem Gemeinschaften sich neu zusammenstellten, konvergierten die Plots auf ähnliche Stufen von phylogenetischer Diversität (Kapitel 10). Die Korrelation zwischen Arten-Kookkurrenz und phylogenetischer Distanz entwickelte sich mit der Zeit ausgehend von anfänglichem phylogenetischem Clustering (Arten sind enger verwandt als man durch Zufall erwartet) zu phylogenetischer Überdispersion. Die Aufschlüsselung der Muster der Kookkurrenz und der phylogenetischen Distanz gab die verstärkte Klumpenbildung von eng und

entfernt verwandten Arten zu erkennen. Dabei exprimierten unterschiedliche Abstammungslinien gegensätzliche Muster der Koexistenz, was darauf hindeutet dass unterschiedliche Stufen von phylogenetischer Verteilung die Interaktionen innerhalb einer Abstammungslinie steuern könnten. Basierend auf grossen Beobachtungsdatenbanken fanden wir, dass bestimmte Typen von Pflanzengemeinschaften sich aus weniger phylogenetischen Abstammungslinien zusammensetzten (in Plots ohne gebietsfremde Pflanzenarten) und dass diese zunehmend gefährdet in Bezug auf invasive gebietsfremde Arten waren. Die Zugabe letzter erhöhte die Verteilung der meisten Merkmale (Kapitel 11). Gemeinschaften mit einer höheren Anzahl an gebietsfremden Arten wiesen auch eine höhere funktionelle Redundanz auf, welche generell mit zunehmender Artenvielfalt erhöht wurde, was wiederum eine erhöhte Ähnlichkeit von Merkmalszuständen aufzeigte (Kapitel 12). Koexistenz von Arten ähnlicher Merkmalsausprägung suggeriert gemeinsame – im Gegensatz zu aufgeteilter – Ressourcennutzung, was darauf hindeutet dass nischenbasierte Mechanismen allein nicht die Koexistenz erklären können, welches wiederum die Rolle und Funktion natürlicher Feinde (z.B. Pathogene) oder Mutualisten in der Gestaltung und Strukturierung von Pflanzengemeinschaften bekräftigt.

Ein Vorbehalt dieser Forschungsarbeit ist die Einschränkung des experimentellen Designs; wir beobachteten die Rückkoppelung von Biodiversität auf Ökosystemfunktionen, allerdings ohne eine Anzahl an natürlichen Prozessen in Betracht zu ziehen. Dennoch konnten wir dadurch unser grundlegendes Verständnis von Artinteraktionen und wie diese ökologische Prozesse steuern verbessern. Ich habe die verschiedenen Art und Weisen wie gemeinschaftsbildende Prozesse durch Artinteraktionen gesteuert werden können dargelegt. Diese Interaktionen sind sehr stark durch die Evolutionsgeschichte bedingt, welche bestimmt wie Artenvielfalt biotische Interaktionen beeinflusst. Ich habe gezeigt dass Gemeinschaftsbildung ein komplementärer Prozess ist, wobei dieser nicht nur anhand von Darlegungen basierend auf Ressourcen erklärt werden kann. Nischenkomplementarität, zusammen mit unserem wachsenden Verständnis der Pathogenregulierung, hat unser Verständnis der entscheidenden biologischen Steuerungsgrössen von gemeinschaftsbildenden Prozessen, wie sie in der vorliegenden Arbeit präsentiert werden, erweitert.

Die Untersuchung gemeinschaftsbildender Prozesse entlang von Biodiversitätsgradienten ermöglicht weitere Erkenntnisse über die Auswirkungen welche der Artenverlust auf Ökosystemfunktionen haben wird. Änderungen in Ökosystemfunktionen überschreiten Schwankungen der Produktivität einer Gemeinschaft und ziehen sich über zentrale biogeochemische Prozessen bis quer durch verschiedene trophische Ebenen. Erhöhte Artenvielfalt verstärkt gleichermassen funktionelle und kompositionelle Vielfalt, die sowohl Ökosystemprozesse als auch die grüne Basis, welche Organismen höherer trophischer Ebenen trägt, stabilisieren. Die wachsenden Belege für den Stellenwert von phylogenetischen Messgrössen suggerieren weiterhin wie Artenvielfalt in hohem Maße wertvolle Informationen, in Bezug auf erbliche Schwankungen, welche wir zum jetzigen Zeitpunkt noch nicht messen können – wie zum Beispiel Pathogenassoziierung – , einbezieht. Über den Beitrag zum fundamentalen Verständnis wie Gemeinschaften und Ökosysteme strukturiert sind und agieren hinausgehend, haben diese Ergebnisse unmittelbare und tiefgreifende Implikationen für Ökosystemmanagement, Rekultivierung und nachhaltige Landwirtschaft. Vor dem Hintergrund der drohenden Gefährdung von Artenvielfalt durch wandelnde Klimabedingungen, intensivierte Landnutzung und die Ausbreitung gebietsfremder invasiver Arten, unterstreicht diese Forschung insbesondere ein Grundsatzprinzip: Biodiversität ist von entscheidender Bedeutung.

General introduction

Biodiversity loss is undeniable (Chapin et al. 2000, Secretariat of the Convention on Biological Diversity 2010) and is the cause of considerable ecological concern (Vitousek et al. 1997, Sanderson et al. 2002, Balvanera et al. 2006). This concern is based on the understanding that species richness and other metrics of biodiversity impact how ecosystems function and how resilient they are to degradation (Naeem et al. 1994, Tilman & Downing 1994, Tilman et al. 1997, Hector et al. 1999, Loreau et al. 2001). We are beginning to understand, in some ecosystem types, which ecosystem functions are most responsive to biodiversity loss (Hooper et al. 2005, Balvanera et al. 2006, Allan et al. 2013). Yet uncertainty remains about the generality of patterns (Balvanera et al. 2006, Allan et al. 2013), which components of biodiversity are the most important and how many species must be lost before ecosystem functioning is radically changed (Ehrlich & Ehrlich 1981, Lawton 1994, Schulze & Mooney 1994, Gitay et al. 1996, Bengtsson 1998, Petchey & Gaston 2006).

Plant community assembly describes the processes by which plant communities come to persist at a given site (Diamond 1975, Lawton 1987). Because plant communities almost always contain more than one species, then they must also be comprised of species interactions that determine the ability of plants to coexist. While many varied coexistence mechanisms have been suggested, the nature and intensity of species interactions can provide insight into those that are most influential (Connell 1978, Shmida & Ellner 1994, Lawton 1987, Tilman et al. 1997, Wilson 1990, Chesson 2000, Adler et al. 2007, Levine & HilleRisLambers 2009). By examining the effect of reducing the number or nature of these interactions, we gain insight into how species loss will impact communities.

In this thesis I examine how community assembly processes vary across biodiversity gradients and what these patterns reveal about coexistence mechanisms and the impact of species loss.

Biodiversity-ecosystem functioning relationships

Biodiversity declines are not limited to species loss, but include reductions of richness on many levels, such as the loss of genetic diversity across populations, decreasing stability in ecosystems, and lower functional diversity within communities (Tilman &

Downing 1994, Naeem et al. 1999). Any analysis of the relationship between biodiversity and ecosystem functioning could therefore be founded on several aspects of diversity, species number, number of functional groups, and presence of particularly important individual species or indices of diversity (Bengtsson 1998, Roscher 2004).

Concern over biodiversity loss has resulted in three decades of experiments, many ongoing, that contribute to what is now a canon of research linking biodiversity and ecosystem-functioning (Balvaneera et al. 2006, Allan et al. 2013). Despite a number of criticisms concerning unrealistic biological assumptions (Huston et al. 2000, Leps 2004), the use of synthetically assembled communities has become the dominant protocol to examine this relationship. And the majority of such experiments manipulate richness in grasslands using different metrics of productivity as a proxy for many ecosystem functions (Diaz et al. 2003).

If species richness has a positive effect on ecosystem functioning then we would expect to see, for example, an increase in community productivity. Such an outcome could result from complementarity, facilitation, or sampling/selection effects (Tilman et al. 1996, Loreau & Hector 2001). Complementarity occurs when interspecific differences in resource requirements or differences in spatial and temporal resource and habitat use results in increasing productivity with increasing species rich communities (Tilman et al. 2001). Facilitation between species could also generate increased productivity, as we expect more positive mutualistic interactions between species if there are more species. Sampling or selection effects describe the situation whereby a more species rich community has a higher probability of containing, and becoming dominated by, a highly productive species (Loreau & Hector 2001). Over-yielding distinguishes complementarity and facilitation from sampling or selection effects and is quantified as the total biomass production of a mixture of species exceeding the highest yielding of the component species in monoculture (Hector et al. 1999).

The presence of complementarity effects have given rise to a number of other hypotheses about ecosystem consequences of biodiversity. The diversity-sustainability hypothesis posits that more diverse systems capture more of the available resources in a system (Tilman et al. 1996, Caridinale et al. 2007), while the diversity-stability hypothesis posits that the greater trait variation in more diverse communities makes

them more likely to contain species that can endure any given environmental disturbance (Tilman & Downing 1994). The insurance hypothesis and the portfolio effect are similar, suggesting that high biodiversity or species richness is not critical for maintaining ecosystem processes under constant environmental conditions, but biodiversity provides a buffer against environmental fluctuations due to varying species responses (Tilman 1999, Yachi & Loreau 1999).

The first results of synthetic assemblage experiments emerging from the Ecotron (Silwood Park, United Kingdom) experiment and the Cedar Creek (Minnesota, United States) field site demonstrated that species rich communities have higher productivity (Naeem et al. 1994, Tilman et al. 1996). Increasing species richness in these systems was also shown to increase the stability of primary productivity (Tilman & Downing 1994) and be more resilient against ecosystem disturbance (Tilman et al. 1996). By looking at different components of the biodiversity gradient, researchers could also show that functional richness rather than species richness played a greater role in driving ecosystem processes (Tilman et al. 1997). New experiments emerged after this first wave of research addressing methodological critiques. The Biodepth experiment measured effects across a range of European grasslands identifying general patterns of species loss resulting in a log-linear decline in productivity (Hector et al. 1999). The Jena Experiment, which was used for a number of chapters in this thesis, was likewise established to deal with critiques. The near orthogonal cross of species richness and functional group richness permits more effective isolation of the mechanisms of positive species richness-ecosystem productivity relationships. The larger plot sizes and the intention for long-term monitoring (the experiment has now been running for 12 years) address additional design concerns. Positive biodiversity effects, revealed through over-yeilding, were identified 2 and 6 years after the experiment was established (Roscher et al. 2005, Marquard et al. 2009). Recent meta-analyses suggest varying responses of different ecosystem processes; the Jena experiment, with huge datasets of response variables, provides an excellent opportunity to further assess which processes are effected most (Naeem et al. 1994, Tilman et al. 1997, Balvaneera et al. 2006, Allan et al. 2013).

Community assembly

Plant community assembly is a hierarchical combination of stochastic and deterministic processes, evident at different levels of community organisation (Weiher & Keddy 1995, Wilson 1999, Leps 2004, Fukami et al. 2005). A hypothetical immigrant must first join the appropriate regional species pool and overcome dispersal barriers to reach a new site (Lawton 1987, Ejrnaes et al. 2006). The composition of regional species pools is determined by biogeographic barriers and other dispersal constraints, as well as rates of evolutionary and extinction processes (Zobel 1997, Zobel et al. 1998, Medail & Diadema 2009).The dispersal component of assembly is generally regarded to be a stochastic process (Hubbell 2001, Myers & Harms 2009); however the sequence of arrival has been shown to exert a strong influence over the resulting community composition (Eriksson & Eriksson 1998, Ejrnaes et al. 2006). Following arrival at a site a species is then subject to abiotic filtering and biotic interactions (Fukami et al. 2005). Abiotic filtering prohibits immigrants unable to establish and reproduce under the local environmental conditions (Keddy 1992, Diaz et al.1998, Myers & Harms 2009).

The biotic filter, which encompasses all the interactions between an immigrant and the local community, is the most interesting part of the process for community ecologists (Lawton 1987). Community assembly must be examined from two, not mutually exclusive angles, when considering how the biotic filter will drive assembly processes. Either community patterns are generated from correlations between species that have a shared or opposite response to their environment, or patterns are subject to assembly rules (Diamond 1975, Wilson 1999). The complexity of influences on assembly processes makes deciphering assembly rules a difficult task. Here I consider an assembly rule to be a limitation on the presence or abundance of a given species due to the presence or abundance of another species or group of species. There are some good examples of assembly rules general enough to permit prediction of community composition (Diamond 1975, Keddy 1992, Wilson 2007, Petermann et al. 2008). The upshot of biotic filtering is that an immigrant can only establish in a community if they can successfully compete with the incumbent resident species (Lawton 1987, Fargione 2003, Stubbs & Wilson 2004, Turnbull 2005), resist the

pressure of local pathogens, pests and herbivores (van der Putten 1997, Bever 2003, Petermann et al. 2008), or find any necessary obligate mutualists (Weiblen et al. 2006).

Coexistence

The nature of the interaction between species is dependent on which coexistence mechanisms are operating in a community. There has been much debate about whether or not coexistence is a niche based or a neutral process (Hubbell 2005, Adler et al. 2007, Levine & HilleRisLambers 2009). Niche differences are theoretically stabilizing mechanisms (Chesson 2000, Adler et al. 2007). If species have niche differences then intraspecific competition will be more intense than interspecific competition and species will limit individuals of their own species more than others leading to stabilized coexistence (Chesson 2000, HilleRisLambers 2009, Turnbull 2014). If communities are structured by niches then competition should lead to the exclusion of species that share similar trait states via competitive exclusion (Gause 1934) which would limit the similarity of species in a community (Macarthur & Levin 1967) as the intensity of their competitive interaction results in the degree of niche overlap being intolerable (Pianka 1974). Such niche based theories suggest that the more similar two species are, the more intense competition between them will be.

Recently a number of researchers have experimentally addressed what species interactions reveal about coexistence mechanisms (Fargione et al. 2003, Von Holle & Simberloff 2004, Turnbull et al. 2005, Emery 2007, Emery & Gross 2007, Mwangi et al. 2007, Petermann et al. 2008, von Felten et al. 2009). Taking a functional group approach, these studies examined if assembly rules operate at a functional level, inferring niche structured coexistence (Fargione et al. 2003, Turnbull et al. 2005, Mwangi et al. 2007, Petermann et al. 2008). By examining how immigrant species perform in resident communities containing the same (home) or different (away) functional groups, it is possible to detect functional group complementary assembly processes. Increased immigrant success in away communities suggest that coexistence is permitted by either avoiding resource-niche overlap and/or a negative feedback resulting from the accumulation of pathogens (Fargione et al. 2003, Turnbull et al. 2005, Mwangi et al. 2007, Petermann et al. 2008).

Evolutionary imprint

Evolutionary history can have a significant impact on community assembly. Species belonging to the same genus often share ancestral ecological traits and environmental preferences ("niche conservatism"; Prinzing et al. 2001, Ackerly 2003, Losos 2008, Cahill et al. 2008, Proches et al. 2008, Thuiller et al. 2010). These similarities promote the chances of closely related species successfully establishing together, as they are more likely to succeed in niches that resemble those to which both are better adapted (Ackerly 2003, Thuiller et al. 2010). Given our understanding of community assembly, the sorting effect of abiotic filtering will be contingent on phylogeny where niche conservatism operates. In the absence of competition, abiotic filtering should cause communities to converge under common abiotic conditions with species belonging to the same genus co-occurring more often than expected by chance (Pfisterer et al. 2004, Fukami et al. 2005). However, niche theory predicts that closely related species should be less likely to coexist, assuming that their shared environmental preferences cause them to compete more intensely (Diamond 1975, Proches 2008, but see Mayfield and Levine 2009).

The impact of additional biotic players (pathogens or mutualists) could also be subject to an evolutionary imprint. The impact of phylogenetic signal across mediated interactions (interactions with additional biotic players) indicates conservatism of host use by biotic go-betweens. The degree of host conservatism for different groups has been shown to vary (Vandenkoornhuyse et al. 2003, Weiblen et al. 2006, Agrawal 2007, Fontaine 2009, Futuyma & Agrawal 2009, Gossner et al. 2009) but there is some evidence that mutualists are increasingly generalists — conserved at higher taxonomic ranks, for example plants from the same family — while pest groups are more commonly host or genus specific (Weiblen et al. 2006). If this distinction holds any sway then we would expect an increased likelihood that species belonging to the same family could co-occur, as they share mutualists. Conversely, species belonging to the same genus would be less likely to co-occur as they share pests. Mediated interactions, like direct competition, could also be density dependent. Generally, the impact of phylogenetic proximity might be density dependent. Abiotic filtering of phylogenetically conserved niches should exert a consistent influence on species regardless of

abundance, but biotic interactions will primarily affect dominant or abundant species that are more likely to compete with a closely related species simply because their abundance dictates an increased chance of contact.

Thesis outline

In **chapter 1** we reviewed what biodiversity experiments reveal about biodiversity–ecosystem functioning relationships. We focused on variation in methodology, collated key publications and synthesized results in order to summarize the key findings in this relationship.

In **chapter 2** we examined the variation in how plant species richness and functional group presence impacts different types of ecosystem processes. We analyzed 418 ecosystem response variables from the Jena Experiment that represent 38 broader ecosystem processes. We calculated the standardized correlation coefficient Zr for the effect of plant species richness, or specific functional group presence for each of the 418 measures. We then analyzed Zr values for species using the different ecosystem process categories in order to test hypotheses regarding how species richness impacts different ecological compartments (above/below ground), biogeochemical cycles and trophic levels.

In **chapter 3** we remained in the Jena Experiment and examined how a generally unreported component of the plant community responds to the species richness gradient. Four and six years after the establishment of the biodiversity gradient we examined how bryophyte communities have assembled along it. We examined how bryophyte species richness responds to vascular plant species richness and how specific functional groups of vascular plants influence the bryophyte community.

In **chapter 4** we looked into the role of pathogen regulation in grassland systems, or so-called Janzen-Connell effects. We removed soil from 3 year old monoculture plots from our Zurich biodiversity platform. In the greenhouse we planted all species on their own (home) soils, and on other (away) soils, and crossed this treatment with an interspecific competition treatment. We applied four soil treatments to different replicates; the treatments were chosen in order to allow us to identify the cause of any home or away soil effects. Treatments included sterilization (gamma irradiation to remove all biota), activated charcoal to remove potential allelochemicals, fertilizer and fungicide addition. We modeled the effect sizes that we identified in order to determine their importance for maintaining diversity.

In **Chapter 5** we returned to the field. Prior to the soil removal in chapter 4, we added seeds from 48 species into established grassland communities with (home) or without (away) a resident species belonging to the same functional group. Our goal was to identify whether or not complementary assembly patterns might emerge, which could suggest either niche complementarity or pathogen regulation. We measured seedling counts and biomass of immigrant species in order to assess any differences in patterns of immigration versus establishment. Resident cover and biomass were measured as covariates. The home/away contrast was crossed with diversity (1 or 3 species) and a nutrient treatment was applied (N, P, or N+P, plus a control) to allow us insight into the role of soil fertility.

In **Chapter 6** we used the home/away effect sizes identified in chapter 4 to examine if alien plant invasion could be regulated by pathogens. We depressed the competitive ability of resident species when they occupy sites formerly occupied by the same species, and then examined the probability and timescale of invasion. We also examined the effect that increasing resident community species richness has on community invasion resistance in a pathogen regulated system.

In **chapter 7** we examined the predictability of community assembly processes as plant communities re-assemble. In established communities in the Jena Experiment, across the species and functional group richness gradient, we added the full species complement to subplots in all plots. In a second subplot we suspended the management regime and permitted stochastic immigration from the regional species pool. For three years we followed the re-assembly process in order to determine the importance of assembly rules versus neutrality. We used our two treatments to assess the role of dispersal limitation.

In **chapter 8** we took a similar approach to the previous chapter but focused on the resident community as opposed to immigrating species. Across the species and functional group richness gradient, we followed the changes in the abundance of sown species in subplots with and without weed management. For 5 years we assessed the impact that immigrating species have on the abundance and the stability of resident species and the role of the species richness.

In **chapter 9** we return our focus to the subplots of chapter 7 but examined the development of phylogenetic patterns. If communities exhibit a phylogenetic signal of clustering we would expect abiotic filtering to be driving the assembly process. However community phylogenetic overdispersion could indicate that biotic interactions are limiting the similarity of species. In the weeded subplots we examined the development of phylogenetic signal in the abundance distributions of species. In the plots with seed addition we examined both phylogenetic dispersion and the response of community phylogenetic diversity.

In **chapter 10** we looked more closely at the correlation between species co-occurrence and phylogenetic distance as it develops with time. We break down these patterns and examine the expectation of linearity in the co-occurrence and phylogenetic distance relationship. In separating the relationship for different lineages we ask the question of whether or not different levels of phylogenetic dispersion could drive species interactions within a lineage.

In **chapter 11** we utilized large databases to extract different plant community metrics. Specifically we targeted the proportion of alien species in a community and measurements of functional traits. We assessed the phylogenetic richness of communities and how this impacts the proportion of alien species present within them. We examined coexistence predictions by analyzing variation in trait states.

In **chapter 12** we leave the European continent and head south to New Zealand. We defined an index for a standardized measure of functional redundancy – the presence of functionally similar species in a community. In 15 communities, with a gradient of species richness, we assessed the level of redundancy evident in 6 leaf traits and examine the implications this has for understanding niche structure.

Breakdown of chapter contributions:

Chapter 1: research; writing

Chapter 2: data collection

Chapter 3: research; design; data collection; analysis; writing

Chapter 4: research; design; data collection; analysis; writing

Chapter 5: research; design; data collection; analysis; writing

Chapter 6: research; design; data collection; writing

Chapter 7: research; design; data collection; writing

Chapter 8: data collection; writing

Chapter 9: research; design; data collection; writing

Chapter 10: research; design; data collection; analysis; writing

Chapter 11: research; writing

Chapter 12: research; design; data collection; analysis; writing

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Chapter 1

Biodiversity Experiments: What Have We Learnt About Biodiversity – Ecosystem Functioning Relationships?

Fergus, A.J.F. & Schmid, B. (2010) *Atlas of Biodiversity Risk* (eds. J. Settele, R. Grabaum, V. Grobelnick, V. Hammen, S. Klotz, L. Penev, I. Kühn). Pensoft, Sofia, Moscow. Chapter 2: pp. 28-31.

Background to biodiversity experiments

Concerns over biodiversity loss have triggered nearly two decades of experiments contributing to a canon of research linking biodiversity and ecosystem functioning. General anxiety regarding biodiversity loss relates to its magnitude across the globe and to the potential consequences on the goods and services that ecosystems provide humanity (Balvanera et al. 2006). More specifically, the concerns of ecologists are focused on how biodiversity losses will impact ecosystem properties such as productivity, carbon storage, and nutrient cycling.

How to investigate the role of biodiversity?

Three main methods have been used to investigate the effect of biodiversity on ecosystem functioning: monitoring studies, field removal experiments and experiments using artificial assemblages of species (Figure 1) (Diaz et al. 2003). These methods can be grouped based on contrasting assembly processes. Both monitoring studies and field removal experiments are carried out in natural communities, incorporating important natural processes. The biodiversity and composition of natural communities is determined by dispersal, the ability to establish under local environmental conditions (abiotic filtering), and by the interaction of incoming species with the biotic community (biotic filtering). In contrast, artificially assembled communities are usually put together by random draw from an experimental species pool. However, this pool is usually carefully selected to include only species that would naturally occur in the same community (Schmid & Hector 2004). There are concerns that random assembly – which translates into random extinction – underestimates the effect of natural processes, and contrasts typically non-random extinction patterns (Leps 2004). The influence of random assembly must be taken into account, but only by directly manipulating species richness under constant abiotic factors can specific ecosystem responses be attributed to changes in biodiversity. Artificial assemblage experiments therefore focus on the feedback from biodiversity to ecosystem functioning. The majority of such experiments manipulate terrestrialplant communities, the basis for a number of fundamental ecosystem processes (Balvanera et al. 2006). Aboveground productivity is the common metric for measuring ecosystem function, it provides a good proxy for services such as

carbon storage, but is not a surrogate for all ecosystem functions. Grasslands are typically used as model ecosystems because they are easily manipulated and productivity can be measured by mowing, which corresponds to either the normal management regime or grazing by herbivores. This contribution focuses on experiments that have manipulated species richness in artificial grasslands, where productivity responses gauge biodiversity effects.

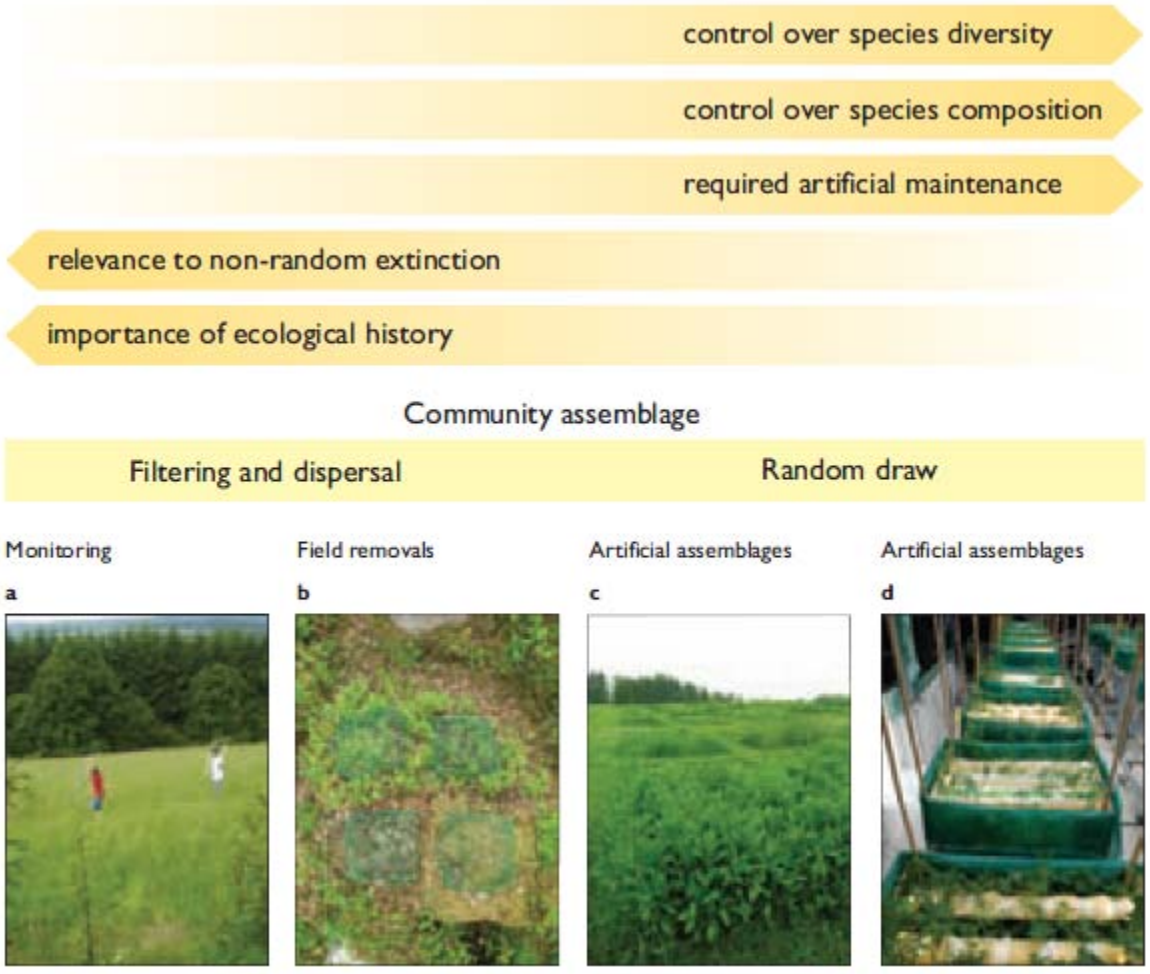

Figure 1. Comparing different approaches to studying biodiversity–ecosystem functioning relationships (modified from Diaz et al., 2003). (A) Monitoring studies in the BIOLOG project (Franconian Forest, Germany); (B) field removal experiments on boreal island ecosystems (Lakes Hornavan, Sweden); artificial assemblage experiments in the field (C) and in microcosms (D) (Zürich, Switzerland). Photos: Juliane Specht (A), Alexander Fergus (B, C), and Yann Hautier (D).

Using artificially assembled communities

Biodiversity has been manipulated at many scales, with different species pools, at various locations around the world. The set up of the Jena Experiment (one of the largest artificial assemblage experiments) provides a good example of the approach (Figure 2). The Jena Experiment was established in 2002 to investigate the effect of biodiversity on element cycling and trophic interactions (Roscher et al. 2004). The Jena Experiment species pool is comprised of 60 plant species common to Central European Arrhenatherion grasslands, artificial communities range in richness from 1-16 species, and contain between 1 and 4 functional groups (Figure 3). Species richness increases on a logarithmic scale: 1, 2, 4, 8, and 16 species, and nearly all possible combinations of species richness x functional group composition occur in the experiment (Figure 4). The composition of each of the plots is maintained by intensive weeding and occasional herbicide application.

Figure 2. The Jena Experiment on the floodplain of the Saale river, Thuringia, Germany (Photo: Jena Experiment consortium).

Figure 3. A high diversity 16-species plot in the Jena Experiment (Photos: Jena Experiment consortium (main image) and Alexander Fergus (inset image).

	Species number																								
F. Group	1	1	1	1	2	2	2	2	2	2	2	2	4	4	4	4	4	4	4	4	4	4	4	4	4
grasses																									
small herbs																									
tall herbs																									
legumes																									
Replicates	4	4	4	4	2	2	2	2	2	2	2	2	1	1	1	1	1	1	1	1	1	1	1	1	4

	Species number																								
F. Group	8	8	8	8	8	8	8	8	8	8	8	8	8	16	16	16	16	16	16	16	16	16	16	16	16
grasses														x16											
small herbs																									
tall herbs															x16										
legumes																									
Replicates	1	1	1	1	1	1	1	1	1	1	1	1	4	1	1	1	1	1	1	1	1	1	1	1	4

Figure 4. The species richness and functional group composition of the large plots of the Jena Experiment. Plant symbols: grasses, small herbs, tall herbs, and legumes (modified from Roscher et al. 2004).

Species richness-productivity theory

If increasing species richness positively affects productivity, then we expect both to increase together. Conversely, reductions in species richness should lead to declines in productivity. Sampling/selection effects and complementarity effects are two general mechanisms proposed to explain this relationship, and respectively relate to single- vs. multi-species processes (Cardinale et al. 2007). The net biodiversity effect is the combination of the two. Sampling/selection effects are species-specific impacts on biomass, thought to occur when the most productive species have a greater chance of being included and eventually dominating the biomass of species-rich polycultures (Cardinale et al. 2007). The terms sampling and selection are often used interchangeably, but the sampling process is shared by both selection and complementarity effects (Loreau & Hector 2001). A community with increased species richness is more likely to contain either single species with particular trait values (selection effects) or a group of species with complementary traits (complementarity effects). Complementarity effects can be seen as the portion of the net biodiversity effect not attributable to any single species. Niche complementarity suggests that greater productivity with increasing species richness results from differences between species in resource requirements, and spatial and temporal resource and habitat use (Tilman et al. 2001). But complementarity effects also include the balance of all forms of niche partitioning that might impact biomass, and all forms of indirect and non-additive species interactions (Cardinale et al. 2007).

Experimental results

One of the first artificial assemblage experiments manipulated both plant and animal biodiversity by creating microcosms of low, intermediate and high species richness (Naeem et al. 1994). These microcosms, housed in the Ecotron system of controlled environmental chambers, revealed that species-rich communities consumed more CO2 than species poor communities and produced more plant biomass. This trend of increased productivity with species richness was also found at the Cedar Creek field site, a nitrogen poor Minnesota grassland (Tilman et al. 1996). Experiments at Cedar Creek have also shown increased species richness to increase both soil nutrient use

efficiency (more sustainable nutrient cycling) and stability of primary production (Tilman et al. 1996). Plant biodiversity was experimentally manipulated in a number of ways at Cedar Creek, but it was increasing species richness and functional group composition that emerged as the major determinants of increasing productivity (Tilman et al. 2001).

The Biodepth experiment increased the generality of these results by testing the biodiversity-productivity relationship across a range of European grasslands (Hector et al. 1999). Results from 8 sites across 7 countries demonstrated that decreasing species richness resulted in a log-linear decline in productivity, whereby reductions in complementarity effects appeared to be responsible (Figure 5). These results deepened the debate over whether complementarity effects or selection effects were generating positive species richness-productivity relationships. In response, Loreau & Hector (2001) devised a method using additive partitioning to separate the two effects. When the Biodepth experiment was re-analysed using this partitioning method, complementarity effects were shown to be positive overall.

Following the first decade of artificial assemblage experiments, designs were adapted to address methodological criticisms. Claims that positive species richness-productivity relationships are dependent on legumes were rejected as assemblages without legumes also detected positive relationships (van Ruijven & Berendse 2003) and complementarity effects were found between species belonging to non-legume functional groups (Loreau & Hector 2001, Tilman et al. 2001). Concerns over random assembly have been addressed with two stage experiments that first delimit the species pool by inducing experimental extinction (Schmid & Hector 2004). By first applying high-intensity management as an extinction filter, the productivity of the resulting species poor assemblages were shown to decrease almost as much as in randomly assembled communities (Schläpfer et al. 2005).

In experiments conducted over a longer period, the positive species richness-productivity relationship increases, and complementarity effects have a progressively greater impact on ecosystem functioning (Tilman et al. 2001). This is supported by a recent metaanalysis summarising 44 experiments where plant species richness was manipulated (Cardinale et al. 2007). On average across these 44 experiments, polycultures produced 1.7 times more biomass than monocultures, and were more

productive in 79 % of experiments. Transgressive overyielding – which describes how the total biomass of a polyculture exceeds that produced by the highest yielding component species in monoculture – was found to occur in only 12 % of experiments (Cardinale et al. 2007). Transgressive overyielding can only result from complementarity effects; hence, positive net effects of biodiversity without overyielding have sometimes been interpreted as evidence for selection effects. But lack of transgressive overyielding does not necessarily conflict with positive species complementarity. Estimates suggest it takes the most diverse polyculture 1750 days before transgressive overyielding begins (Cardinale et al. 2007). Because most experiments run for an average of 730 days, it is likely that complementarity effects have so far been underestimated.

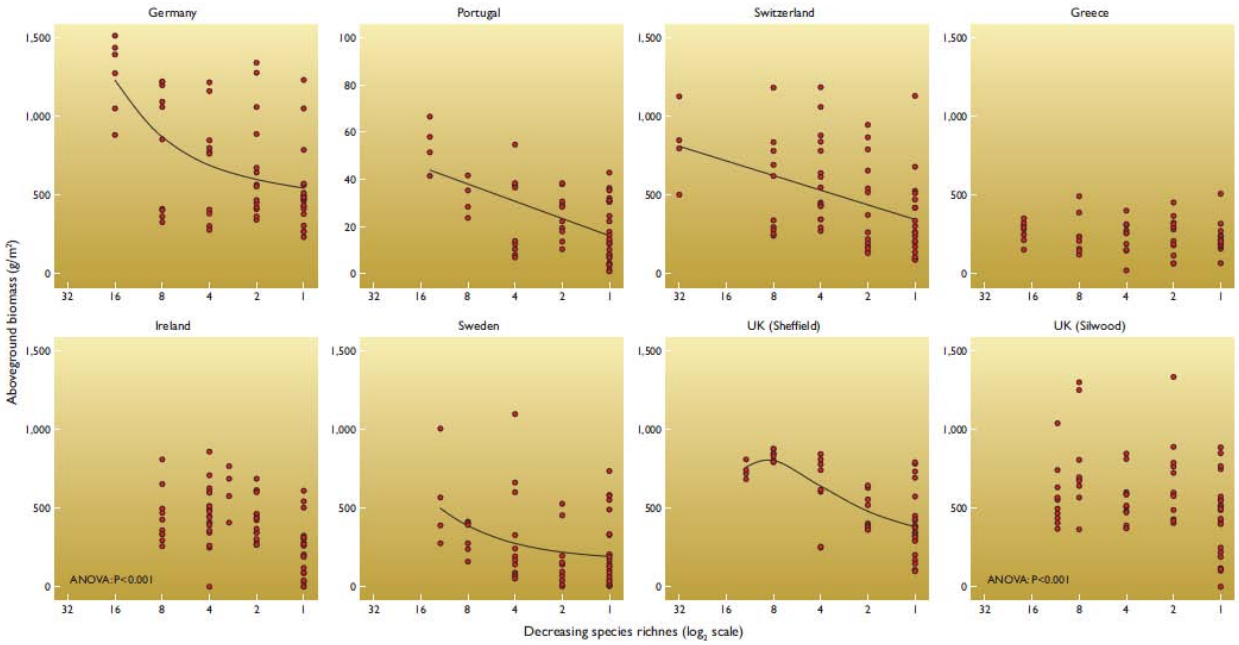

Figure 5. Biomass patterns at each of the Biodepth sites, species richness is on a log2 scale. Best-fit models from individual sites based on adjusted R2 are as follows: log-linear in Switzerland and Portugal; linear (untransformed species richness) in Germany and Sweden; quadratic in Sheffeild; ANOVA with five species richness levels (significant treatment effects with no simple trend) in Ireland and Silwood; and no significant effect in Greece (Source: Hector et al. 1999).

Current directions

Because biodiversity spans a range of biotic scales, from genetic variation within a species to biome distribution across the planet, recent experiments have explored other measures of both biodiversity and ecosystem functioning. This is a necessary step, as maintenance of an increasing number of ecosystem processes has been shown to require more species (ecosystem multifunctionality) as different species often influence different ecosystem functions and processes (Hector & Bagchi 2007). Plant-pollinator interactions represent another key ecosystem function where biodiversity has recently been manipulated across trophic levels (Figure 6) (Fontaine et al. 2006). Manipulating the functional diversity of both plants and pollinators was shown to increase recruitment of more diverse plant communities. Complementarity between functional groups is thought to have generated the result, which suggests that functional diversity of pollinator networks may well be critical to ecosystem stability (Fontaine et al. 2006). Recent results from the Jena Experiment also expand our understanding of which ecosystem functions and processes respond most to changes in biodiversity. Analyses of 418 variables revealed carbon measures to be influenced more by species loss than variables associated with the nitrogen cycle, reiterating the role of biodiversity in mitigating climate change (Allan et al. 2013).

To include another measure of biodiversity, the genetic diversity of populations of a single species has recently been manipulated (Crutsinger et al. 2006). The genotype diversity of Tall Goldenrod, *Solidago altissima*, was manipulated by creating populations with the same number of individuals but containing 1, 3, 6, or 12 genotypes (Figure 7). Aboveground productivity increased with plant genotype diversity, and was 36 % higher in 12- genotype vs. single-genotype plots (Figure 8). Extending beyond the productivity function, a positive relationship was also found between genotype diversity and the diversity of associated consumers. The number of arthropods was on average 27 % higher in 12-genotype vs. single genotype plots, and not simply because of increased plant productivity (Figure 8). Most recently ecologists have asked how the evolutionary

Figure 6. Manipulating biodiversity across trophic levels; cages containing different functional diversity combinations of both plants and pollinators. Inset: monitoring pollinator behaviour. Photos: Colin Fontaine.

Figure 7. Populations of Tall Goldenrod, *Solidago altissima*, assembled so that each population is made up of 1, 3, 6, or 12 genotypes. Inset: sampling arthropod diversity as a response to Tall Goldenrod genotype diversity. Photos: Gregory Crutsinger.

relationships among species predict how biodiversity impacts productivity (Cadotte et al. 2008). The phylogenetic diversity of communities was found to explain more variation in plant productivity than species richness. Therefore in artificial assemblages there is a greater effect of biodiversity on productivity when plant species are more distantly related to one another (Cadotte et al. 2008).

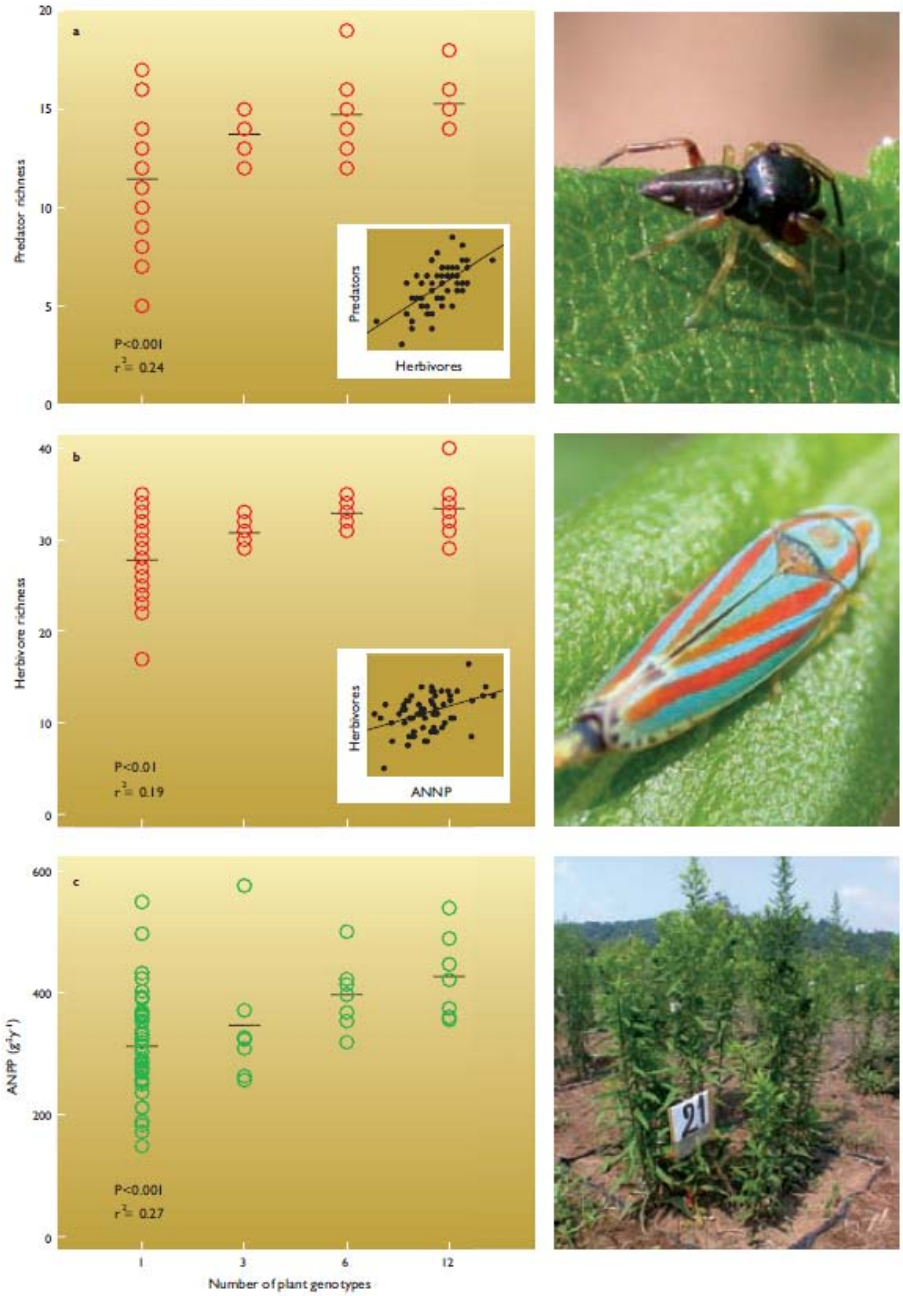

Figure 8. Increased arthropod biodiversity (a, b) and plant productivity (c) in response to increasing genotype diversity of Tall Goldenrod, *Solidago altissima* (Source: Crutsinger et al. 2006). Photos: Gregory Crutsinger.

Biodiversity conclusions

Across countries, species pools, evolutionary histories, and even within the genetic code of a single species, evidence suggests that biodiversity has significant impacts on the production of biomass and associated ecosystem processes (Hector et al. 1999, Crutsinger et al. 2006, Cadotte et al. 2008). As a result of manipulating community species richness and functional group composition, increased biodiversity has been shown to positively impact nutrient retention, soil sustainability and carbon cycling (Tilman et al. 1996, Allan et al. submitted). But there are limitations to artificial assemblage experiments; we are seeing the feedback from biodiversity to production, but without the incorporation of most natural processes (Schmid & Hector 2004). We now have a good idea how grassland systems operate, and the role of biodiversity within them, but the incorporation of natural processes may still generate unexpected results. More long-term experiments are required in grasslands and experiments in general must expand into other systems dominated by species with different life forms and life histories. Encouragingly, biodiversity experiments with tree species are underway in Borneo, China, France, Finland, Germany, and Panama, but such systems will take time to generate results. More and different response variables must also be measured, as focus on individual processes may underestimate the biodiversity necessary for ecosystem functioning (Hector & Bagchi 2007). These recommendations echo the most recent species richness-productivity results, which suggest if anything, we may have underestimated the impact of species richness and in turn species loss on ecosystem functioning.

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Chapter 2

A comparison of the strength of biodiversity effects across multiple functions.

Allan, E., Weisser, W.W., Fischer, M., Schulze, E-D., Weigelt, A., Roscher, C., Baade, J., Barnard, R.L., Beßler, H., Buchmann, N., Ebeling, A., Eisenhauer, N., Engels, C., Fergus, A.J.F., Gleixner, G., Gubsch, M., Halle, S., Klein, A.M., Kertscher, I., Kuu, A., Lange, M., Le Roux, X., Meyer, S.T., Migunova, V.D., Milcu, A., Niklaus, P.A., Oelmann, Y., Pašalić, E., Petermann, J.S., Poly, F., Rottstock, T., Sabais, A.C.W., Scherber, C., Scherer-Lorenzen, M., Scheu, S., Steinbeiss, S., Schwichtenberg, G., Temperton, V., Tscharntke, T., Winfried Voigt, W., Wilcke, W., Wirth, C. & Schmid, B. (2013) A comparison of the strength of biodiversity effects across multiple functions. *Oecologia* **173**: 223-237.

Abstract

In order to predict which ecosystem functions are most at risk from biodiversity loss, meta-analyses have generalised results from biodiversity experiments over different sites and ecosystem types. In contrast, comparing the strength of biodiversity effects across a large number of ecosystem processes measured in a single experiment permits more direct comparisons. Here, we present an analysis of 418 separate measures of 38 ecosystem processes. Overall, 45 % of processes were significantly affected by plant species richness, suggesting that, while diversity affects a large number of processes not all respond to biodiversity. We therefore compared the strength of plant diversity effects between different categories of ecosystem processes, grouping processes according to the year of measurement, their biogeochemical cycle, trophic level and compartment (above- or belowground) and according to whether they were measures of biodiversity or other ecosystem processes, biotic or abiotic and static or dynamic. Overall, and for several individual processes, we found that biodiversity effects became stronger over time. Measures of the carbon cycle were also affected more strongly by plant species richness than were the measures associated with the nitrogen cycle. Further, we found greater plant species richness effects on measures of biodiversity than on other processes. The differential effects of plant diversity on the various types of ecosystem processes indicate that future research and political effort should shift from a general debate about whether biodiversity loss impairs ecosystem functions to focussing on the specific functions of interest and ways to preserve them individually or in combination.

Introduction

Understanding the relationship between biodiversity and ecosystem functioning is of great theoretical interest for understanding the processes structuring communities, and of practical importance to predict the effect of human-induced biodiversity loss. Numerous experiments have demonstrated that a range of ecosystem functions depend on biodiversity (usually species richness) (Hector et al. 1999; Loreau et al. 2001; Tilman et al. 2001; Hooper et al. 2005). In addition, certain key functional groups, such as grasses and legumes in grassland ecosystems, can also have large effects on

ecosystem functioning (Hooper et al. 2005). However, it is still not clear which particular ecosystem variables are most strongly affected by species richness or functional group composition. This question is important as it relates to our understanding of the mechanisms that underlie the biodiversity–ecosystem functioning relationship. For biodiversity research to be predictive, it is therefore necessary to move forward from showing that biodiversity has an effect on functioning to investigating which functions are most strongly affected.

Recently, meta-analyses and syntheses have attempted to answer this question by comparing the strength of biodiversity effects on different processes, across different experiments (Balvanera et al. 2006; Cardinale et al. 2006, 2011; Schmid et al. 2009; Hooper et al. 2012). This generalises across sites; but processes measured in different experiments may not always be directly comparable. An alternative approach is to synthesize data from a single experiment and to investigate the effect of biodiversity on different processes measured on the same plots (Proulx et al. 2010; Scherber et al. 2010; Rzanny and Voigt 2012). This has the advantage that different variables and ecosystem functions can be directly compared, without being affected by variance between experimental sites. We therefore use this approach here and present a large analysis of results from a German biodiversity experiment, the Jena Experiment (Roscher et al. 2004). We include 418 measures of 38 ecosystem processes.

The length of time an experiment has been running is likely to be an important factor affecting the strength of biodiversity effects found. Biodiversity effects have been shown to become stronger over time, as complementary interactions between species become more important in long-term experiments (Cardinale et al. 2007), resulting in less saturating relationships between biodiversity and function (Reich et al. 2012). Studies have so far focussed on individual variables such as biomass production and it is not clear if this pattern holds across a wider range of ecosystem processes.

The interactions between carbon, nutrient and water cycles are fundamental to ecosystem functioning (Schulze and Zwölfer 1994), and it is therefore important to know whether they are affected differently by biodiversity loss. Loss of biodiversity has been shown to reduce biomass production (Hector et al. 1999; Tilman et al. 2001; Marquard et al. 2009), and affect other pools and fluxes of the carbon (Hooper et al. 2005;

Fornara and Tilman 2008; Steinbeiss et al. 2008) and nitrogen cycle (Tilman et al. 1996; Scherer-Lorenzen et al. 2003; Hooper et al. 2005; Palmborg et al. 2005; Oelmann et al. 2011). A relationship between plant biomass production and nutrient uptake would be expected in ecosystems strongly limited by nutrients where resource-use complementarity for nutrients may be the dominant mechanism driving the species richness–biomass relationship (Tilman et al. 2001). However, resource-use complementarity for nutrients might not be so important in productive systems or those limited by factors other than nutrient availability, for instance, if plant enemies and not nutrients limit biomass production in low diversity communities (Maron et al. 2010; Schnitzer et al. 2011). In such systems, plant diversity might have large effects on biomass production and carbon cycling but smaller effects on nutrient uptake and other measures of nutrient cycling.

As well as potential differences between biogeochemical cycles, plant diversity effects might also vary between other classes of ecosystem process. Plant diversity has been shown to have a larger effect on above- than belowground animal groups in the Jena Experiment (Scherber et al. 2010), and this may be because belowground organism groups respond more slowly (Eisenhauer et al. 2010) or in a more idiosyncratic fashion to plant diversity (de Deyn and van der Putten 2005). Broadening the scope beyond organism groups, belowground processes in general might be less strongly affected by plant species richness than are aboveground processes because the belowground processes are principally microbially-mediated and therefore less directly affected by plants (Hooper et al. 2005). Similarly, plant diversity might have larger effects on direct (biotic) measures of other organism groups than on abiotic measures, which are mediated by, but which are not direct measures of, organisms. In particular, strong effects of plant species richness on direct measures of animals, such as the abundance and diversity of insects, are to be expected due to co-evolutionary interactions between plants and animals (e.g. Haddad et al. 2009; Eisenhauer et al. 2011), but this might not be true for plant species richness effects on abiotic processes more indirectly associated with organisms such as biogeochemical cycling. Finally, the contrast between measures of fluxes and measures of standing stocks has been suggested as a major distinction between types of functions (Pacala and Kinzig 2001).

Many of these contrasts, between biogeochemical cycles, above- and belowground variables and biotic and abiotic variables, will be at least partially confounded, for instance many nutrient measures are likely to be abiotic and belowground. Therefore, only a large analysis with many measures of each category of process can determine which contrasts are the most important for predicting differences in plant diversity effects.

Understanding the effect of changes in plant diversity for other trophic levels is important for predicting the impact of plant species extinctions on total biodiversity. A previous synthesis of results from the Jena Experiment (Scherber et al. 2010) investigated the effects of plant species richness on the abundance and diversity of other trophic levels and found that the response of different organisms to plant diversity varied strongly. Herbivores were more likely to show a significant response to plant species richness than were predators, parasitoids or omnivores. This suggests strong bottom-up effects on multitrophic interaction networks and shows that plant diversity effects on higher trophic levels are indirectly mediated through bottom-up trophic cascades. Plant species richness might also have larger effects on animal species richness than on abundance, if rare animal species are only present in diverse plant communities. The analysis by Scherber et al. (2010) showed this pattern for a number of invertebrate groups. More generally, plant species richness might have its strongest effects on the diversities of other groups of organisms. Here, we extend the analysis of Scherber et al. (2010) by including a larger number (418) of measures of ecosystem processes that come from all compartments of the ecosystem, i.e. our dataset is not restricted to measures of animal groups. For instance, in the comparison of plant species richness effects between trophic levels, we include the producer trophic level and, when comparing plant species richness effects between diversity and other measures, we additionally test whether plant species richness has a stronger effect on measures of animal diversity than on measures such as plant biomass production. We can therefore test whether the patterns of stronger plant diversity effects on herbivores and on the species richness of animal groups hold when the analysis is extended to include a wider range of ecosystem processes.

In addition to effects of plant species richness on ecosystem processes, the presence of key plant functional groups may be important for driving certain functions. It has been suggested that soil processes such as decomposition, nutrient uptake and nutrient retention are affected more by the functional traits of dominant species than by species richness per se (Hooper et al. 2005). Functional composition, and the presence of legumes in particular (Vitousek and Howarth 1991; Temperton et al. 2007), could therefore have a larger effect on nutrient cycling than plant species richness does.

To investigate variation in the strength of plant species richness and functional group effects between different types of ecosystem processes, we grouped measured variables into a number of categories (Table 1) associated with basic ecological processes. For each of the measures analysed here, we quantified the effect size of species richness and functional group (legume and grass) presence using Zr values (Balvanera et al. 2006). We then analysed the Zr values for species richness and presence of legumes and grasses using the ecosystem process categories (Table 1) as explanatory terms (Balvanera et al. 2006; Schmid et al. 2009). We tested the following hypotheses:

1. **Plant species richness effects increase in strength over time.**
2. **Plant species richness has stronger effects on carbon than on nutrient cycling.**
3. **Plant species richness has larger effects on processes measured above- than belowground.**
4. **Plant species richness has strong bottom-up effects on higher trophic levels and these are larger on lower trophic levels (herbivores vs. carnivores).**
5. **Plant diversity has its strongest effects on the species richness of animal groups.**
6. **Functional groups such as legumes and grasses have stronger effects on nutrient cycling than plant species richness does.**

Table 1: The explanatory terms used in the analysis

Ecosystem process term	Categories
Biogeochemical cycle	Carbon: variables that are principally carbon, i.e. biomass and abundance measures, carbon concentrations, and CO_2 and CH_4 emission rates
	Nutrients: measures of nutrient concentrations in the soil and in plant biomass, N-related enzyme activities in soil, N_2O emission rates, 15N signals
	Water: measures of soil water
Trophic level	Producer: measures of plants
	Herbivore: abundance and species richness of herbivore groups (including pollinating insects and foliar fungal pathogens) and measures of herbivory
	Decomposer: abundance and species richness of decomposer groups
	Carnivore: abundance and species richness of carnivorous groups
	Ecosystem: abiotic measures
Compartment	Above: all measures taken aboveground
	Below: all measures taken belowground
Diversity versus other processes	Diversity: measures of animal and pathogen species richness
	Other processes: all other measures
Abiotic versus biotic	Abiotic: all abiotic measures; i.e. those which are not direct measures of organisms but can include processes affected by organisms, such as soil nutrient levels
	Biotic: all biotic measures; i.e. those which are direct measures of organisms such as plant biomass or plant nutrient concentrations
Static versus dynamic	Static: measures of pool sizes
	Dynamic: measures of fluxes

Six ecosystem process terms were used to group all 418 measurements into the categories shown. In addition to these terms, year and soil depth of measurement were included as continuous fixed terms

Materials and methods

Experimental design

The measurements reported here were gathered between 2002 and 2008 in the Jena Experiment, a grassland biodiversity experiment in Germany which controlled the number of plant species, functional groups and plant functional identity in 82 plots, each 20 9 20 m, in a randomized block design. Plants belonged to one of four functional groups (for details, see Roscher et al. 2004): legumes, grasses, tall herbs and small herbs and the presence/absence of these functional groups was manipulated factorially with species richness. Thus, the design included communities of single functional groups with 1–16 species as well as communities of 16 species ranging from 1 to 4 functional groups. In our analyses, we focus on the effects of legumes and grasses, because many studies have identified these as important functional groups and because the herb functional groups might not be comparable with groups in other grasslands. Plots were seeded in May 2002 with 1, 2, 4, 8, 16 or 60 perennial grassland plant species, with 16, 16, 16, 16, 14 and 4 replicates, respectively. Plot compositions were randomly chosen from 60 plant species typical for local *Arrhenatherum* grasslands. Plots were maintained by mowing, weeding and, where possible, by applying grass- or herbspecific herbicides, all twice per year (Roscher et al. 2004).

The dataset

We included 418 measurements of ecosystem processes in our analysis. All measurements were taken independently, i.e. none of the measurements are direct functions of other measures. The 418 measures were nested within 119 variables and these variables were nested within 38 ecosystem processes (see Table S1). The ecosystem processes were in turn nested within 6 larger categories of processes, such as carbon- versus nutrient-related processes (shown in Table 1). These groups were partially crossed with each other, e.g. carbon variables could be measured above- or belowground and could be biotic or abiotic. Our analysis tested for differences between these larger groups. In order to conduct a global analysis, all variables were classified

according to these 6 categories of processes. As the assignment of certain variables, such as plant biomass, to a particular biogeochemical cycle is not trivial, we further analysed a smaller dataset composed of measures that could be unambiguously assigned to one or another cycle, see below. Many of the 119 variables had been measured in multiple years and/or at multiple soil depths, and we included all these multiple measures in our analyses in order to test for trends in the strength of effects over time and soil depth. However, we used mixed modelling to account for the nestedness of measurements and the spatial and temporal autocorrelation of variables; see below. Most processes and variables were measured between 2003 and 2006 (2002, 6 and 9; 2003, 21 and 48; 2004, 23 and 45; 2005, 19 and 58; 2006, 20 and 38; 2007, 13 and 21; and 2008, 1 and 8 processes and variables, respectively).

Statistical analysis

Deriving Zr values and significances for the individual measures

We calculated effects of plant species richness, or the presence of functional groups, on each of these 418 measures as the standardized correlation coefficient *Zr*, an effect-size value often used in meta-analysis (Gurevitch and Hedges 1999). *Zr* values were extracted from analysis of variance (ANOVA) models using the following formula:

ANOVA model (Eq. 1)

block + log(species richness) + legumes + grasses + tall herbs + small herbs

r values were calculated as the proportion of total sum of squares explained by species richness, legume or grass presence and were converted with a *Z*-transformation to improve normality, using the formula (Rosenberg et al. 2000):

$Zr = 0.5\ 1n\ ((1 + r)/(1 - r))$

Sequential (type I) sums of squares were used (Schmid et al. 2009), which means effects of legumes were corrected for species richness and effects of grasses were corrected for species richness and legumes. According to the design of the Jena Experiment, these explanatory factors are as nearly as possible, but not perfectly, orthogonal to each other (Roscher et al. 2004). All analyses were conducted using the statistical package R 2.14 (R Development Core Team 2010).

Comparing diversity effects between different categories of ecosystem process

To compare different categories of process, we then analysed *Zr* values, related to plant species richness and functional group effects, as a function of the ecosystem process categories in Table 1. This analysis is essentially a derived variable analysis and is therefore equivalent to a repeated measures analysis using the original data. It is also similar to a meta-analysis in which data taken from a single experiment are analysed to show differences among within-experiment explanatory terms but is different from standard meta-analysis conducted on data from many experiments. Here, each particular ecosystem process category (for instance, all measures related to the carbon cycle) is represented by several variables which can be considered as independent replicate measures for the purpose of comparing between different groups within the ecosystem process category (e.g. comparing carbon and nitrogen measures). However, unlike in a typical metaanalysis, but as in all experimental studies, our conclusions will, of course, only apply to this one experiment.

Mixed modeling

Linear mixed-models (fitted using the lme4 package Bates et al. 2011 in R) were used to analyse the *Zr* values. The different ecosystem process categories presented in Table 1 were used as fixed explanatory terms. We used random effects to account for the nestedness of our data: measures nested within ecosystem variables and ecosystem variables within ecosystem processes. Mixed models included ecosystem variable identity as a random effect with 119 levels (variable in model formula; column 2 in Table S1). Crossed with this term were random effects for year and soil depth (many soil measures were taken at different depths; all aboveground measures were coded as

0 depth). Ecosystem process (Fig. 1) was included as a random effect with 38 levels, and we also included the interaction between ecosystem process and year as another random effect; this had 109 levels. In order to test for temporal or spatial trends in the *Zr* values, we included linear contrasts for year and soil depth as fixed terms. We also conducted a jackknife analysis (see below) to check that our results were robust to any additional sources of non-independence between measures. As some measures were only taken on a subset of plots, the *Zr* values were also weighted by the proportion of plots on which the original measure was taken.

All fixed terms (the explanatory terms in Table 1 as well as year and soil depth) were fitted both individually and in a combined analysis, i.e. they were removed from the full model (Eq. 2) and added to the minimal model (Eq. 3). As a conservative test, we only considered fixed effects significant if they were significant in both cases, i.e. when added to the null model and when removed from the full model. We used these stringent rules because the fixed effects were not fully orthogonal to each other and we wanted to ensure that our conclusions would hold both if an explanatory term of interest was, or was not, corrected for other, correlated explanatory terms. Significance for each term was assessed by model comparison using likelihood ratio (L-ratio) tests (Crawley 2007). In addition, significance of terms was assessed using Markov Chain Monte Carlo sampling (Baayen et al. 2008), for terms fitted in the full model, which did not change the significance of any terms. The full and null models (using the syntax of the lme4 package; Bates et al. 2011) are shown below; see Table 1 for a description of the fixed effect terms and Table S1 for the assignment of variables to the different fixed and random effect terms:

Full model (Eq. 2):

year (linear) + soil depth (linear) + biogeochemical cycle + trophic level + diversity others + abiotic biotic + compartment + static dynamic + (1| variable) + (1| soil depth) + (1| year) + (1| ecosystem process) + (1| ecosystem process: year)

Minimal model (Eq. 3):

Intercept + (1| variable) + (1| soil depth) + (1| year) + (1| ecosystem process) + (1| ecosystem process: year)

where "(1|…)" indicates the random effects, the model estimates the variance between the means for each level of the random effect (all random effects are categorical here).

Further analyses with biogeochemical cycle

In order to explore species richness effects on different biogeochemical cycles further, the analysis was restricted to variables that were direct measures of carbon, nutrients or water. This analysis, therefore, excluded variables such as plant biomass or animal abundances, which could be associated with multiple biogeochemical cycles (see Table S1 for list of excluded variables), and was conducted with 67 carbon measures, 83 nutrient measures and 38 water measures. Equation 2 was used to fit these models but without the terms "TrophicLevel" and "DiversityOthers", as there were no measures of animals included. We also repeated this analysis including aboveground pool sizes of carbon and nitrogen in plant tissue (shoot and root), instead of measures of carbon and nitrogen concentrations in plant biomass. Pool size is calculated as concentration × plant biomass. Note that we included concentrations and not pool sizes in the main analysis, because pool sizes are closely correlated with plant biomass and would therefore not be independently measured variables, as they represent linear combinations of concentrations and biomass.

Differences between carbon (C) and nutrient (N) cycles could be due to differences in the size or in the sign of the *Zr* values. For some variables, it could be argued that a negative sign indicates a positive effect of diversity on function. It is clear that a positive correlation between species richness and biomass equates to a positive effect on function, but in other cases this might not be straightforward. For instance, lower soil nitrogen levels might correspond to increased plant nitrogen uptake, which would be associated with an increase in functioning. However, lower soil nitrogen might also result from a decreased mineralization rate, which would imply a decrease in

functioning. To avoid these problems, we analysed *Zr* values with their original sign in the main analysis. However, we conducted additional analyses in which we varied the sign. Firstly, we repeated the analysis with the sign reversed for soil N variables: if the main difference between C and N variables is that N variables are significantly negatively affected by plant species richness whereas C variables are significantly positively affected, this analysis would show no difference between the two. Secondly, as a more conservative test, we repeated the analysis of direct measures of carbon, nutrients and water, including pool sizes rather than concentrations and reversing the sign for all those ecosystem variables that had a negative mean *Zr* value (these were: soil nitrate, soil δ15N values, soil phosphorus, plant δ15N values and methane oxidation). Therefore, in this analysis, all ecosystem variables analysed had a positive mean *Zr* value, although clearly some of the individual measures of each ecosystem variable were still negative. If there are certain variables which are significantly negatively affected by plant diversity (such as soil nitrate where a negative value could indicate high functioning), and if these drive the difference between C and N cycles, they would be significantly positively affected in this analysis and again the difference between C and N cycles would disappear. Note that it is not possible to analyse absolute *Zr* values because this would inflate effect sizes. Ecosystem variables that are not significantly affected by diversity should on average have a *Zr* value of zero, corresponding to a mix of slightly positive and slightly negative *Zr* values for the different measures. Absolute *Zr* values would mean ecosystem variables always had a positive mean *Zr* value and thus would appear to be correlated with diversity even if they were not.

A larger number of carbon-related measures (294) had been taken compared with nutrient-related measures (83) or water-related measures (41). To assess whether this unequal sampling affected the significance of the biogeochemical cycle term, the number of carbon and nutrient-related measures was equalised with the number of water-related variables by randomly selecting 41 carbon-related and 41 nitrogen-related measures. This process of jackknifing also provides a much more conservative test, as only 123 measures are included instead of 418. The analysis was repeated 1,000 times

with different sets of randomly selected carbon and nutrient variables using the following formula:

Jackknife model (Eq. 4):

year (linear) + soil depth (linear) + biogeochemical cycle+ (1| variable) + (1| soil depth) + (1| year) + (1| ecosystem process) + (1| ecosystem process: year)

Significance of the term biogeochemical cycle was therefore assessed by comparing models fitted with Eq. 4 to models fitted with Eq. 3, using L-ratio tests.

Results

Across all processes, species richness had on average a positive effect (mean effect size ± 1SE = 0.08 ± 0.05; this is the intercept from a linear mixed model without any fixed effects (Eq. 3) and is therefore corrected for the random effects). To determine the proportion of ecosystem processes significantly affected by plant species richness, confidence intervals were calculated around the mean Zr value for each of the 38 ecosystem processes (see Fig. 1). Of these, 17 had confidence intervals which did not cross 0, suggesting that nearly half (45 %) of processes were on average significantly affected by species richness.

Change in species richness effects over time and soil depth

The linear terms for year and soil depth were significant in the analysis of species richness *Zr* values: the slope for year was positive (0.026 ± 0.008) indicating an increase in the magnitude of *Zr* values, and thus in the effects of species richness, over time from 0.02 in 2002 to 0.19 in 2008 (Fig. 2a). Plant species richness effects increased over time significantly for plant biomass, soil water contents and the abundance of decomposers and marginally so for soil nitrate Fig. 3a. Plant species richness effects decreased significantly over time for the abundance of carnivores and marginally so for the abundance of herbivores. The slope for the soil depth term was negative (−0.0022 ± 0.0007), indicating a decrease in the strength of the species

richness effect with increasing soil depth (Fig. 2b). Plant species richness effects decreased with soil depth significantly for soil water and soil nitrate (Fig. 3b).

Differences between ecosystem processes categories

Two of the ecosystem process categories showed significant overall species richness effects: the biogeochemical cycle and the contrast between diversity measures and measures of other processes (Fig. 1a; Table 2). On average, plant species richness had a significantly positive effect on variables related to the carbon cycle (confidence intervals did not overlap 0) but non-significant overall effects on nutrient- (mostly nitrogen) and water-cycle related variables (Fig. 4a; see also Fig. 1a for the individual processes contained in the categories). Most variables associated with the carbon cycle, including biomass of plants, abundance of animals and soil organic carbon storage, were positively correlated with diversity (see Fig. 1a), while among the water variables species richness effects declined with increasing soil depth so that only water content of the topsoil was significantly positively affected (see Figs. 1a, 2b). In contrast to the overall positive effects on carbon and water variables, most measures related to the nitrogen cycle had small Zr values and their confidence intervals included zero, suggesting zero or small effects of plant species richness on soil nitrogen pools and fluxes (Fig. 1a). The Zr values for species richness effects were also significantly affected by the variable diversity/others, because plant species richness had stronger effects on the diversities of other organisms (0.35 ± 0.09) than on other measures such as animal abundances, stock sizes of abiotic pools, and flux measures (0.06 ± 0.05).

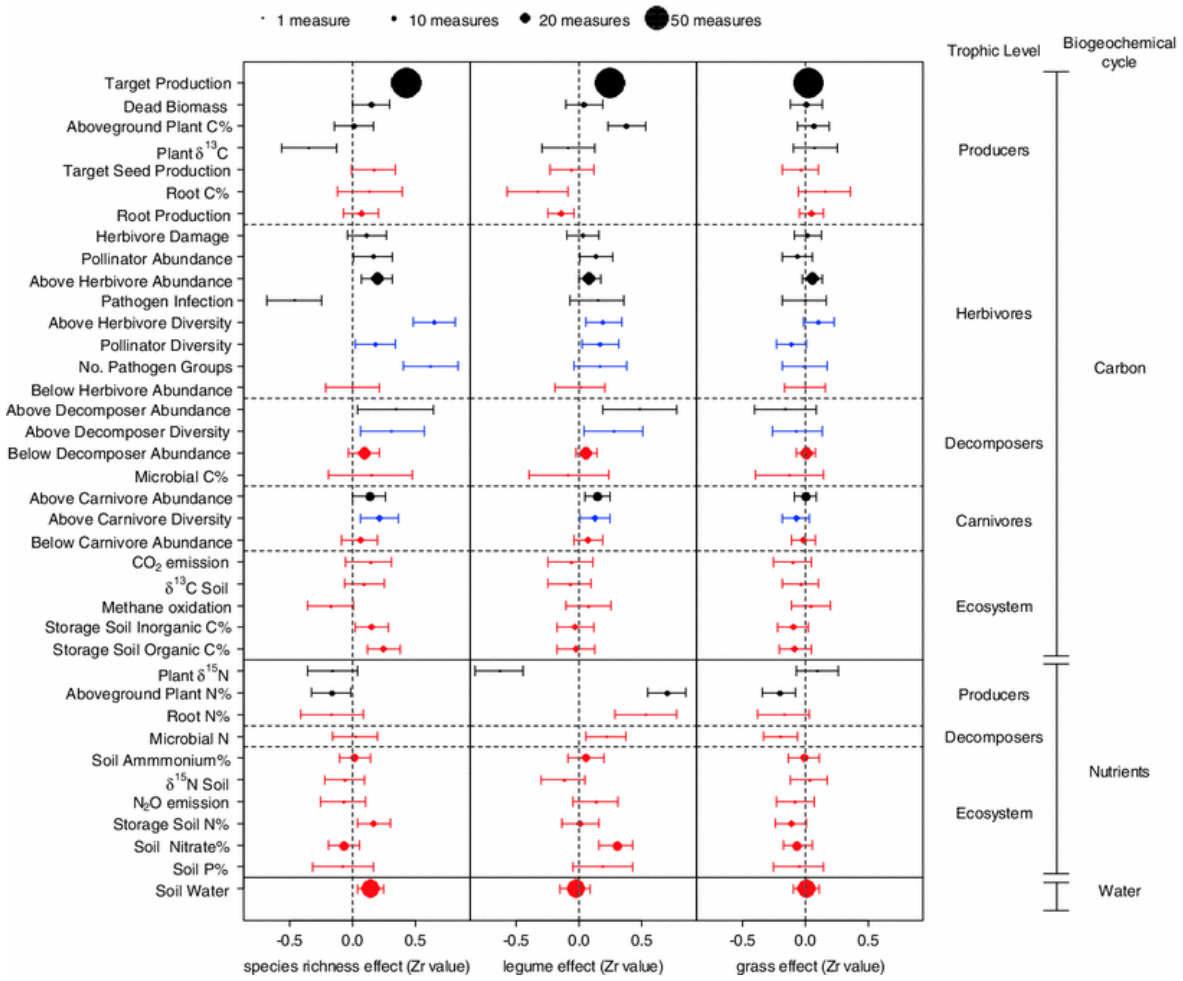

Fig. 1. The effect of **a** species richness and the presence of **b** legumes and **c** grasses on a range of ecosystem processes. All measures have been grouped according to the ecosystem process with which they are associated. Effect sizes, measured as *Zr* values, are shown for the different ecosystem processes with 95 % confidence intervals: ecosystem processes whose confidence intervals do not include 0 can be considered to be significantly affected by species richness or functional group presence. The size of the points is scaled according to the total number of measures taken per ecosystem process. *Points* represent estimates calculated from Markov Chain Monte Carlo (MCMC) sampling of terms from a linear mixed effect model with ecosystem process as a fixed effect and the random effect structure specified in Eq. 3 (“Materials and methods”), MCMC means are very similar to the weighted means. *Error bars* represent 95 % confidence intervals calculated using MCMC sampling. Processes are grouped according to the biogeochemical cycle to which they belong (carbon, nutrient, water); these are separated by *solid lines*. Within the carbon variables, processes are grouped according to trophic level (producer, herbivore, decomposer, carnivore, ecosystem); these are separated by *vertical dashed lines*. Processes in *red* are those measured belowground and those in *black* were measured aboveground. Processes in *blue* are measures of diversity (all of these are also aboveground measures). *C* Carbon, *N* nitrogen, *P* phosphorus.

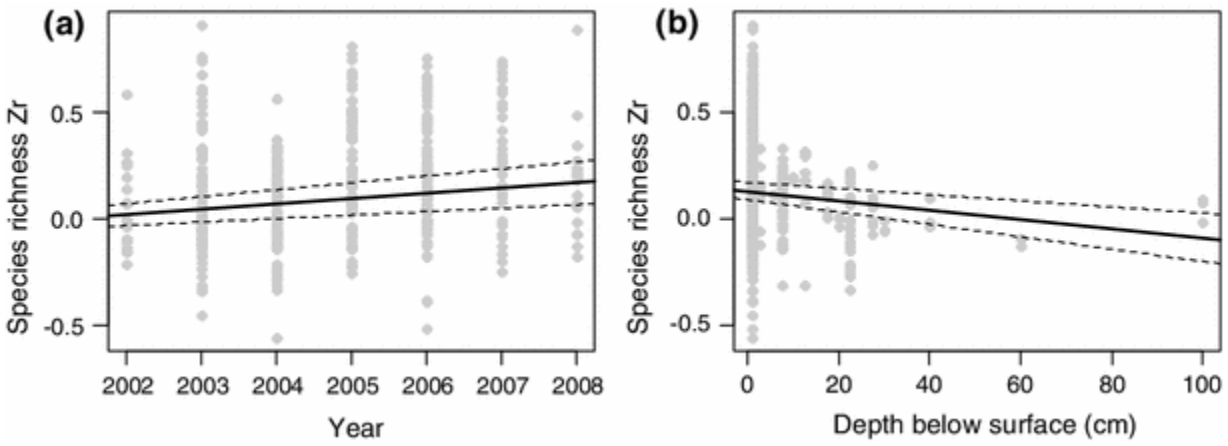

Fig. 2. Change in the size of species richness effects over time and soil depth. Average species richness Zr values are shown for each a year and b soil depth. In both cases the solid line is the prediction from a linear mixed model with the random effect structure in Eq. 2 and with a year and b soil depth fitted as fixed effects. Dotted lines show ±1SE

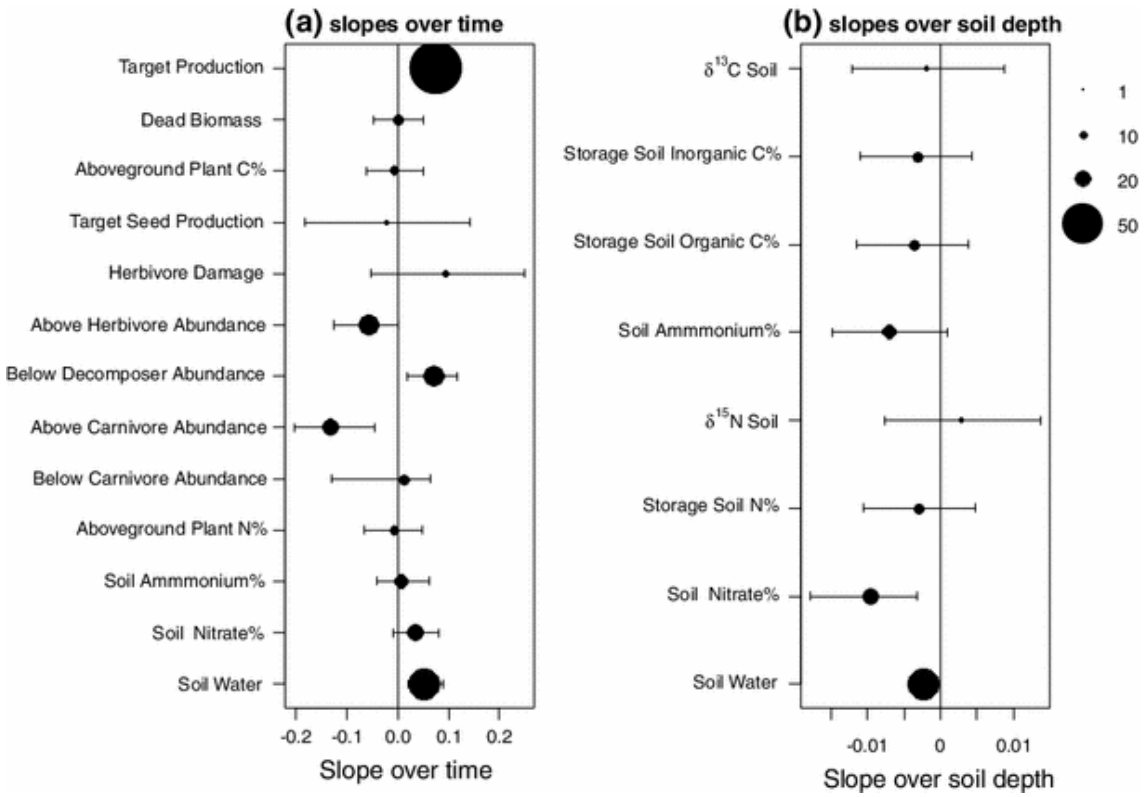

Fig. 3. Slopes showing the change in the strength of species richness effects (*Zr* values) on various ecosystem processes over **a** time and **b** soil depth. All processes which were measured in **a** three or more years and **b** three or more soil depths are shown. Points and 95 % confidence intervals come from Markov Chain Monte Carlo MCMC sampling of mixed models. Mixed models were fitted with fixed effects: ecosystem process, year (in **a**) or soil depth (in **b**) and their interaction, i.e. different slopes were estimated for each ecosystem process. Random effects were variable and the variable × year (factorial) interaction, see “Materials and methods”. Points are proportional to the number of measures taken for each ecosystem process (i.e. number of variables × number of times each variable was measured).

Table 2: The significance of explanatory terms used in the analyses

	Degrees of freedom	**Species richness**		**Legume presence**		**Grass presence**	
		+	**−**	**+**	**−**	**+**	**−**
Year	1	7.4**	7.1**	0.1^{NS}	0.7^{NS}	5.1*	6.8***
Space	1	7.2**	6.7**	0.08^{NS}	0.3^{NS}	1.0^{NS}	$3.3^{0.07}$
Trophic level	4	2.8^{NS}	1.1^{NS}	2.4^{NS}	0.2^{NS}	6.3^{NS}	4.3^{NS}
Biogeochemical cycle	2	6.7*	5.8*	1.2^{NS}	3.4^{NS}	6.1*	3.1^{NS}
Compartment	1	2.4^{NS}	0.5^{NS}	4.2*	2.0^{NS}	1.3^{NS}	0^{NS}
Diversity versus others	1	10.7**	7.1**	1.7^{NS}	0^{NS}	0.6^{NS}	0.7^{NS}
Abiotic versus biotic	1	0.7^{NS}	0.5^{NS}	1.1^{NS}	0^{NS}	1.0^{NS}	0.4^{NS}
Static versus dynamic	1	0.01^{NS}	0.15^{NS}	2.1^{NS}	0.1^{NS}	1.4^{NS}	0.2^{NS}

Explanatory terms were fitted in linear mixed-effects models with Zr values for species richness, legume presence or grass presence effects as response variables (see “Materials and methods” for description of the models). The table shows χ 2 values from Likelihood-ratio tests: the “+” columns are for the explanatory term fitted alone (i.e. added to the intercept only model) and values in the “−” columns are for terms deleted from a model containing all the other explanatory terms (“Materials and methods”). Asterisks indicate significance: *5 %, **1 %, ***0.1 %, ^{NS} non-significant effects; p values >0.05 and <0.08 are shown. Values in bold are those that were significant on deletion and on addition; according to our strict criteria, these are the only terms that are considered significant.

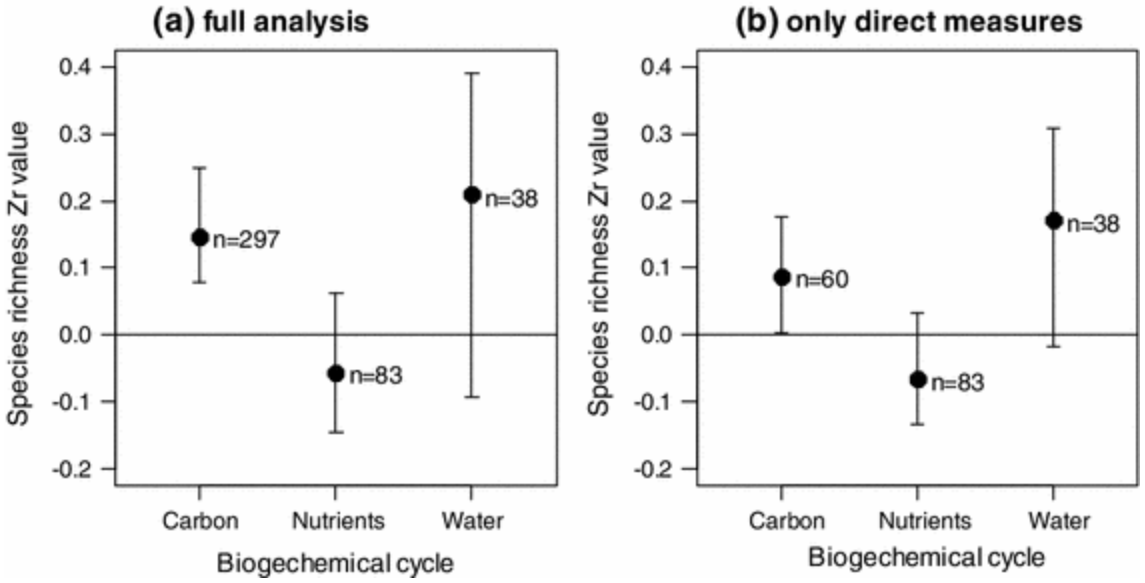

Fig. 4. The mean *Zr* values and 95 % confidence intervals for species richness effects, shown for variables belonging to different biogeochemical cycles. **a** The full analysis with all 418 measures and **b** the reduced analysis with only the 181 direct measures of the different biogeochemical cycles, i.e. excluding those measures, such as plant biomass, which can be associated with more than one of the cycles. Points represent estimates calculated from Markov Chain Monte Carlo (MCMC) sampling of terms from a linear mixed effect model with biogeochemical cycle as a fixed effect and the random effect structure specified in Eq. 2 ("Materials and methods"), MCMC means are very similar to the weighted means. Error bars represent 95 % confidence intervals calculated using MCMC sampling.

Further analyses with biogeochemical cycle

We also carried out a number of sensitivity analyses to explore the differences in the size of species richness effects between different biogeochemical cycles. When only variables that were direct measures of carbon, nutrients or water (i.e. excluding biomass and abundance measures; see Table S1) were included in the comparison between the biogeochemical cycle groups, this resulted in an increase in the significance of the term, from $\chi^2 = 5.8$, $p = 0.03$ with all variables included, to $\chi^2 = 9.1$, $p = 0.01$ with only direct measures (both p values for deletion of the term from the full model; Fig. 4b). In the analysis of direct measures, plant species richness had a significantly positive effect on carbon measures, whereas, overall, plant species richness did not have a significant effect on nutrient measures (Fig. 4b). When aboveground pool sizes of nitrogen and carbon in plant tissue were used instead of concentrations in this analysis, the comparison between groups remained significant on deletion from the full model ($\chi^2 =$

6.5, p = 0.04) and marginally so when biogeochemical cycle was tested on its own (χ^2 = 4.8, p = 0.09). These results together further support stronger species richness effects on the carbon than the nutrient cycle.

When the analysis of *Zr* values was carried out with the sign for the soil nutrient variables reversed, the biogeochemical cycle term was still significant (addition χ^2 = 8.2, p = 0.01; deletion χ^2 = 6.6, p = 0.03). When the sign was reversed for only those soil variables with a negative mean Zr value, biogeochemical cycle also remained significant (addition χ^2 = 6.9, p = 0.03; deletion χ^2 = 8.2, p = 0.02). When direct measures of carbon and nutrients were analysed, using pool sizes rather than concentrations, and with the sign for all variables with a negative mean Zr value reversed, the biogeochemical cycle remained significant when deleted from the full model (χ^2 = 8.2, p = 0.01), although not when tested alone (χ^2 = 2.6, p = 0.27). These results show that the contrast in plant species richness effects between biogeochemical cycles is not caused by a difference in the direction of the effect (e.g. the contrast is not caused by strong negative effects of plant species richness on nutrient measures and strong positive effects of plant species richness on carbon measures) rather the contrast is caused by a difference in the size of the effects, which are stronger for carbon measures and weaker for nutrient measures.

When the analysis of biogeochemical cycles was repeated using equal numbers of carbon-, nutrient- and water-related measures, the biogeochemical cycle term was significant in 836 out of 1,000 runs. This suggests that unequal sampling did not affect the results. It also suggests that the result was robust to a decrease in the degrees of freedom for testing the effect of biogeochemical cycle, as it generally remained significant when only 30 % of the variables were included. This indicates that any additional non-independence between variables, not accounted for by our random effect structure, did not bias the result for the biogeochemical cycle term.

Together, our additional sensitivity analyses on the differences between biogeochemical cycles support larger overall species richness effects on the carbon cycle and small or variable effects on the nutrient and water cycles.

Effects of functional group presence

None of the grouping variables significantly affected the *Zr* values for effects of grasses or legumes (Fig. 1b, c; Table 2), although the strength of grass effects increased with time (slope 0.011 ± 0.004). Comparing the strength of the effects of functional group presence with the strength of species richness effects showed that, for nutrient measures, legume effects were larger than species richness effects: the average *Zr* value for legume effects on nutrient measures was 0.13 ± 0.07 compared to a species richness *Zr* value of −0.05 ± 0.07. Most measures of nutrients increased with legume presence, in particular nitrogen concentrations in plants and microbes as well as the nitrate pool size (Fig. 1b). Grass effects on nutrient measures were also stronger than species richness effects and, contrary to legume effects, were more negative: the average *Zr* value was −0.08 ± 0.04. Grass presence had negative effects on nitrogen tissue concentrations and nitrate pools (Fig. 1c). For carbon measures, species richness effects were larger (0.15 ± 0.05) than were legume (0.07 ± 0.04) or grass (0.008 ± 0.01) effects.

Discussion

Overall, ecosystem processes were positively correlated with plant diversity. The average *Zr* value for species richness effects was 0.08 ± 0.05, slightly higher than the figure of 0.039 reported for grassland studies in a meta-analysis by Balvanera et al. (2006). Our results show that plant species richness effects are on average positive across a wide range of ecosystem processes; however, there was substantial variability in the effects, given the wide range of different ecosystem processes measured. Recent studies have shown that biodiversity effects on biomass can be comparable to the effects of other environmental change drivers (Hooper et al. 2012; Tilman et al. 2012), and it will therefore be important to compare the effects of biodiversity and other environmental change drivers on a larger number of ecosystem processes to understand the relative importance of biodiversity.

We found that around 45 % of ecosystem processes were significantly affected by plant species richness. Plant species richness effects are therefore important for a large number of ecosystem processes, though not all processes respond. It is, however,

possible that simultaneously maintaining high levels of multifunctionality of the other (non-responding) processes would require high plant diversity (Hector and Bagchi 2007; Isbell et al. 2011). We investigate the causes of the large variation in the strength of plant species richness effects between ecosystem processes in order to identify which types of processes respond strongly.

Trends over time

The magnitude of the species richness effect increased since the start of the experiment. Other studies have shown that biodiversity effects on biomass production (Cardinale et al. 2007; Marquard et al. 2009; Reich et al. 2012), on soil nitrogen variables (Oelmann et al. 2011) and on the soil biota (Eisenhauer et al. 2010) become stronger with time. These results agree with ours (Fig. 2a). In addition, we find that plant diversity effects increased over time for soil water content. The soil organisms may have taken several years to colonise the experimental communities, explaining the increasing plant diversity effects over time (Eisenhauer et al. 2011). Different mechanisms are likely to be behind the effects for the other ecosystem processes. Functional redundancy between species has been shown to decrease over time, resulting in less strongly saturating species richness biomass relationships over time (Reich et al. 2012). This may be due to an increase in positive, complementary interactions between species over time, and turnover between functionally dissimilar species (Allan et al. 2011), resulting in greater functional diversity in more mature plant communities (Reich et al. 2012). This in turn may have been associated with greater biomass production as well as reduced water loss from diverse plots. Our analysis shows a strong pattern of increasing biodiversity effects over time for a number of different ecosystem processes.

Differences between biogeochemical cycles

Species richness effects differed between groups of variables belonging to different biogeochemical cycles. On average, we found that plant species richness had significantly positive effects on carbon variables but no significant effects on nutrient measures (mostly nitrogen). Soil carbon storage was increased in species-rich communities perhaps due to both increased plant inputs and increased microbial

respiration (Steinbeiss et al. 2008). A previous meta-analysis of biodiversity effects on function did not find this difference in effect size between biogeochemical cycles (Balvanera et al. 2006), but it has been suggested that changes in vegetation composition may cause imbalance between biogeochemical cycles (Schulze and Zwölfer 1994). Our results suggest that the contrast between carbon and nutrient measures was more important for predicting the strength of plant species richness effects on ecosystem function than was the contrast between abiotic and biotic measures, measures of pools and fluxes or above- and belowground measures. Our analysis therefore suggests that, despite the usual close coupling of nitrogen and carbon cycling, the loss of plant biodiversity may have larger effects on the carbon than the nitrogen cycle.

There are a number of possible reasons for the difference in plant species richness effects between carbon and nutrient cycles. Plant species richness might have larger effects on carbon than nitrogen cycling because overyielding, the increased biomass production of more species-rich communities compared with less diverse communities, was driven by mechanisms other than resource-use complementarity. If the plant species richness biomass relationship is driven by resource complementarity for nitrogen, plant species richness effects on carbon and on nitrogen measures would be expected to be similar. However, direct measurements of belowground niche differentiation have not yet provided strong evidence for resource-use complementarity in diverse mixtures (von Felten et al. 2009). Further, in productive sites, diverse communities may be limited by light competition (Roscher et al. 2011), which causes plants to invest more in N-poor structural tissue (Hirose and Werger 1995), therefore reducing nitrogen concentrations in aboveground biomass in species rich communities. The plant species richness–biomass relationship might also be driven by plant natural enemies, resulting in weaker effects on nutrients than on carbon. Soil fungal pathogens can drive the diversity–productivity relationship by causing large reductions in biomass in species-poor plant communities (Maron et al. 2010; Schnitzer et al. 2011). In low diversity communities, soil pathogens might also reduce rooting volume, therefore reducing uptake of nutrients as well as carbon production (de Kroon et al. 2012). However, aboveground fungal pathogens or herbivores could act in a similar way to

belowground pathogens: infection by foliar fungal pathogens strongly decreases with species richness in our experiment (Fig. 1a). These aboveground enemies could remove substantial quantities of biomass in low-diversity communities (Carson et al. 2004; Allan et al. 2010) and therefore drive the species richness biomass relationship. In general, it may be the case that, where the species richness biomass relationship is driven by niche complementarity for nitrogen, plant species richness has strong effects on both carbon and nitrogen cycling, but if the plant species richness biomass relationship is driven by natural enemies then plant species richness might have relatively weaker effects on nitrogen than on carbon cycling.

Differences between above- and belowground processes

The strength of biodiversity effects decreased with increasing soil depth but, contrary to our expectations, the contrast between above- and belowground processes was not significant. Scherber et al. (2010) found smaller plant species richness effects on belowground invertebrates, but this cannot explain the soil depth effect as belowground organisms were not measured at different depths. Plant species richness has also been suggested to influence microbially-mediated soil processes less strongly than plant-mediated aboveground productivity (Hooper et al. 2005), although this distinction may be less important here as we also find smaller plant diversity effects on root biomass as opposed to shoot biomass (Bessler et al. 2009). We find that processes, such as soil water and nutrient contents measured at greater soil depths, are affected less strongly by plant diversity. Smaller plant diversity effects on nutrients at greater soil depths may result from reduced plant uptake of nutrients or reduced plant inputs to the soil at depths where root biomass is lower (Jackson et al. 1996). The positive plant diversity effects on topsoil water contents (and smaller effects at greater soil depths) probably arise through increased shading and therefore reduced evaporation in diverse plant communities (Rosenkranz et al. 2012). Our results suggest that the above/belowground contrast is therefore less important for predicting the strength of plant diversity effects and that, instead, plant diversity effects decline continuously with increasing soil depth.

Bottom-up effects on higher trophic levels

Our results provide strong evidence for positive bottom-up effects of plant diversity on herbivore, pollinator, pathogen, decomposer and carnivore groups. This result agrees with other, partial, syntheses of the Jena Experiment results (Scherber et al. 2010; Eisenhauer et al. 2011), although, unlike the analysis by Scherber et al. (2010), here we find no consistent differences between plant species richness effects for different trophic levels, which also suggests that our analysis is quite conservative. There are a number of possible reasons for the positive bottom-up effects of plant diversity. A diverse plant community may support a greater diversity of specialist herbivores and/or generalist herbivores might benefit from the increased diversity of plant resources in more species-rich plant communities (resource specialization hypothesis) (Siemann 1998; Haddad et al. 2009). It is also possible that a greater total quantity of resources in diverse plant communities could support a greater number and biomass of herbivore individuals and therefore a greater diversity of species (more individuals hypothesis) (Haddad et al. 2009). The latter hypothesis may be less likely here because we found that the diversities of animal groups were more strongly influenced by plant species richness than were abundances of these animals, which would not be expected if plant diversity primarily increases herbivore abundance and secondarily herbivore species richness. Note that we have no measures of herbivore biomass: a recent analysis provided strong evidence for the more individuals hypothesis but this was mediated by herbivore biomass not herbivore abundance (Borer et al. 2012). The stronger plant diversity effects on animal species richness as compared to animal abundance might be due to a greater number of rare insect species in high diversity plant communities (Haddad et al. 2009). Declining plant diversity should lead to a faster decline in species richness than in total abundance of animal groups if rarer animal species are the first to be affected by plant diversity loss. The especially strong plant species richness effects on the diversities of other organisms imply that ecosystem services which depend on animal diversity, such as provision of natural enemies and pollinators, are likely to be particularly threatened by loss of plant species (Blüthgen and Klein 2011).

Functional group effects

Functional group composition also had strong effects on certain ecosystem processes, in particular those associated with the nitrogen cycle. In general, functional group effects on nitrogen cycling were stronger than species richness effects, even though functional group presence was fitted after species richness in the ANOVA models (see Eq. 1). Our results agree with a number of other experiments, which have shown strong functional group effects (Hooper and Vitousek 1998; Scherer-Lorenzen et al. 2003; Palmborg et al. 2005; Temperton et al. 2007). Most measures of nitrogen increased with legume presence because legumes fix atmospheric nitrogen and therefore increase nitrogen stocks (Craine et al. 2002; Oelmann et al. 2007; Temperton et al. 2007). Grass presence had negative effects on nitrogen measures most likely because grasses are good competitors for nitrogen and deplete soil nutrient pools (Craine et al. 2002; Oelmann et al. 2007). Therefore, whereas the carbon cycle was mainly affected by plant species richness and grass presence, the nitrogen cycle was affected by legume presence and less so by grass presence. This suggests that changes in functional composition should have a larger effect on nitrogen cycling than would changes in species richness.

Conclusions

Our analysis, focused on measures from a single experiment, shows clear patterns of variation among biodiversity effects on a large number of different ecosystem functions. Taken together, our results stress that a wide variety of ecosystem functions will be at risk from local extinctions of plant species, but some will be more sensitive than others. In addition, further studies need to test whether the same ecosystem processes are strongly affected by biodiversity in managed systems where biodiversity responds to environmental change and affects ecosystem function. Our results emphasise the importance of considering a wide set of functions, and a broad range of measures representing those functions, in order to draw general conclusions in biodiversity–ecosystem functioning studies.

Our study indicates that the ability of ecosystems to sequester carbon will be particularly impaired by loss of plant species, as soil carbon storage in the soil was

reduced in low diversity communities (Steinbeiss et al. 2008). Nutrient cycling will probably be less severely affected by plant species loss. In this case, direct effects of nitrogen deposition on nutrient cycling may be more severe than indirect effects mediated through changing species composition (Manning et al. 2006), although a loss of species from the particular functional group of legumes could have strong indirect effects. However, in more nitrogen-limited systems, where the plant species richness–biomass relationship is more likely to be driven by resource complementarity for nitrogen, loss of plant species richness might have larger effects on nitrogen cycling. In general, the strength of plant diversity effects on different types of ecosystem processes might depend on which factor drives the species richness–biomass relationship. Further comparative studies in other systems, comparing the strength of biodiversity effects between multiple processes measured in the same experiment, are needed to test this idea. We therefore hope that our findings stimulate further tests of the mechanisms underlying biodiversity effects in order to better understand variation in the strength of effects between different types of ecosystem processes.

Acknowledgments

We would like to thank Volker Kummer (supervised by M.F.), Peter Mwangi, Maike Habekost (supervised by G.G.), Yvonne Kreutziger (supervised by W.W. and Y.O.) and Ramona Müller (supervised by W.V.) for contributing data. The gardeners and technical staff who have worked on the Jena Experiment, for maintaining the site, weeding, mowing and data collection: Steffen Eismann, Steffen Ferber, Silke Hengelhaupt, Sylvia Junghans, Ute Köber, Katja Kunze, Heike Scheffler and Sylvia Creutzburg, Jens Kirchstein, Olaf Kolle, Gerlinde Kratzsch, Anett Oswald, and Ulrike Wehmeier. We would also like to thank a large number of student helpers who were involved in the weeding of the experiment, Cornelius Middelhoff and Jens Schumacher for maintaining the database and Jean-Francois Soussana and Tania Jenkins for comments on earlier drafts of the manuscript. This project was funded by the Deutsche Forschungsgemeinschaft DFG with additional contributions from the Swiss National Science Foundation (Grant 31003A-107531 to B.S.).

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Table S1: full list of all variables used in the analysis.

The variable name is given, along with the ecosystem process with which it is associated and its assignment to the explanatory variables listed in Table 1. Also shown are the number of measures taken of each of the variables and the number of plots on which the variable was measured (N). To explore the biogeochemical cycle effect further a separate analysis was conducted excluding variables that were not direct measures of carbon, nutrients or water, the variables excluded are indicated in the column "Biogeochemical Cycle Direct Measures" as "excluded". Abbreviations are given for elements: carbon (C) nitrogen (N) and phosphorus (P).

Number of times measured	Variable	Ecosystem Process (Fig 2)	N	Static Dynamic	Trophic Level	Biogeochemical Cycle	Biogeochemical Cycle Direct Measures	Diversity/ Others	Abiotic/ Biotic	Compartment
8	Average vegetation height	Target Production	81	static	producer	carbon	excluded	Material	biotic	above
13	Biomass above target	Target Production	81	static	producer	carbon	excluded	material	biotic	above
13	Cover target	Target Production	82	static	producer	carbon	excluded	material	biotic	above
10	Leaf area index	Target Production	81	static	producer	carbon	excluded	material	biotic	above
5	Mean of maximum height transect flowers	Target Production	75	static	producer	carbon	excluded	material	biotic	above
2	Plant modules target per m²	Target Production	82	static	producer	carbon	excluded	material	biotic	above
13	Prop cover vegetation	Target Production	65	static	producer	carbon	excluded	material	biotic	above
12	Biomass above dead	Dead Biomass	82	static	producer	carbon	excluded	material	biotic	above
11	Plant C%	Plant C%	79	static	producer	carbon	carbon	material	biotic	above
6	Number of propagules target per m²	Target Seed Production	82	static	producer	carbon	excluded	material	biotic	below
2	Fine root C%†	Root C%	75	dynamic	producer	carbon	carbon	material	biotic	below
2	Fine root production	Root Production	79	dynamic	producer	carbon	excluded	material	biotic	below
2	Fine root stock	Root Production	80	static	producer	carbon	excluded	material	biotic	below
2	Large root production	Root Production	79	dynamic	producer	carbon	excluded	material	biotic	below
2	Large root stock	Root Production	80	static	producer	carbon	excluded	material	biotic	below
2	Mean diameter of roots	Root Production	80	dynamic	producer	carbon	excluded	material	biotic	below
1	Mean diameter of standing roots	Root Production	81	static	producer	carbon	excluded	material	biotic	below
2	Root length increment	Root Production	80	dynamic	producer	carbon	excluded	material	biotic	below
1	Mean herbivory *Plantago lanceolata* phytometers	Herbivore Damage	82	static	herbivore	carbon	excluded	material	biotic	above
2	Mean herbivory *Rumex*	Herbivore Damage	82	static	herbivore	carbon	excluded	material	biotic	above

	acetosa phytometers									
1	Mean Herbivory *Trifolium pratense* phytometers	Herbivore Damage	81	static	herbivore	carbon	excluded	material	biotic	above
4	Mean Herbivory along Transect (%)	Herbivore Damage	80	static	herbivore	carbon	excluded	material	biotic	above
1	Mean Mollusc Herbivory *Vicia faba* phytometers	Herbivore Damage	80	static	herbivore	carbon	excluded	material	biotic	above
6	Flower visitor frequency	Pollinator Abundance	76	static	herbivore	carbon	excluded	material	biotic	above
1	Hymenoptera broad cells per plot	Pollinator Abundance	82	static	herbivore	carbon	excluded	material	biotic	above
1	Hymenoptera dead broad cells	Pollinator Abundance	76	static	herbivore	carbon	excluded	material	biotic	above
1	Hymenoptera nests per plot	Pollinator Abundance	82	static	herbivore	carbon	excluded	material	biotic	above
1	Number of Aphidina	Above Herbivore Abundance	82	static	herbivore	carbon	excluded	material	biotic	above
1	Number of Saltatoria	Above Herbivore Abundance	50	static	herbivore	carbon	excluded	material	biotic	above
2	Number of Cicadina	Above Herbivore Abundance	50	static	herbivore	carbon	excluded	material	biotic	above
2	Number of Heteroptera	Above Herbivore Abundance	50	static	herbivore	carbon	excluded	material	biotic	above
1	Number of phytophagous Coleoptera	Above Herbivore Abundance	50	static	herbivore	carbon	excluded	material	biotic	above
1	Number of phytophagous Diptera	Above Herbivore Abundance	50	static	herbivore	carbon	excluded	material	biotic	above
1	Number of phytophagous Diptera (leaf chewers)	Above Herbivore Abundance	50	static	herbivore	carbon	excluded	material	biotic	above
1	Number of phytophagous Diptera (miners)	Above Herbivore Abundance	50	static	herbivore	carbon	excluded	material	biotic	above
1	Number of phytophagous Heteroptera	Above Herbivore Abundance	50	static	herbivore	carbon	excluded	material	biotic	above
1	Number of phytophagous Hymenoptera	Above Herbivore Abundance	50	static	herbivore	carbon	excluded	material	biotic	above
1	Number of Auchenorrhyncha	Above Herbivore Abundance	82	static	herbivore	carbon	excluded	material	biotic	above
2	Number of Diptera	Above Herbivore Abundance	82	static	herbivore	carbon	excluded	material	biotic	above
3	Number of Gastropoda	Above Herbivore Abundance	82	static	herbivore	carbon	excluded	material	biotic	above
2	Number of phytophagous Coleoptera	Above Herbivore Abundance	82	static	herbivore	carbon	excluded	material	biotic	above
2	Number of sucking herbivores	Above Herbivore Abundance	82	static	herbivore	carbon	excluded	material	biotic	above
1	Number of Thysanoptera	Above Herbivore Abundance	82	static	herbivore	carbon	excluded	material	biotic	above

2	Number of vole burrowing holes	Above Herbivore Abundance	82	static	herbivore	carbon	excluded	material	biotic	above
3	Pathogen infection	Pathogen Infection	81	static	herbivore	carbon	excluded	material	biotic	above
2	Species richness Cicadina	Above Herbivore Diversity	50	static	herbivore	carbon	excluded	information	biotic	above
1	Species richness Heteroptera	Above Herbivore Diversity	50	static	herbivore	carbon	excluded	information	biotic	above
1	Species richness phytophagous Coleoptera	Above Herbivore Diversity	50	static	herbivore	carbon	excluded	information	biotic	above
2	Species richness phytophagous Diptera	Above Herbivore Diversity	50	static	herbivore	carbon	excluded	information	biotic	above
1	Species richness phytophagous Heteroptera	Above Herbivore Diversity	50	static	herbivore	carbon	excluded	information	biotic	above
1	Species richness phytophagous Hymenoptera	Above Herbivore Diversity	50	static	herbivore	carbon	excluded	information	biotic	above
1	Species richness Saltatoria	Above Herbivore Diversity	50	static	herbivore	carbon	excluded	information	biotic	above
1	Hymenoptera species per plot	Pollinator Diversity	82	static	herbivore	carbon	excluded	information	biotic	above
1	Hymenoptera species per trap	Pollinator Diversity	82	static	herbivore	carbon	excluded	information	biotic	above
6	Species number of flower visitors	Pollinator Diversity	76	static	herbivore	carbon	excluded	information	biotic	above
3	Pathogen groups	No. Pathogen Groups	81	static	herbivore	carbon	excluded	information	biotic	above
2	Number of phytophagous insect larvae	Below Herbivore Abundance	82	static	herbivore	carbon	excluded	material	biotic	below
1	Number of plant feeding Nematodes	Below Herbivore Abundance	73	static	herbivore	carbon	excluded	material	biotic	below
3	Number of Diplopoda	Above Decomposer Abundance	50	static	decomposer	carbon	excluded	material	biotic	above
2	Number of saprophagous Diptera	Above Decomposer Abundance	50	static	decomposer	carbon	excluded	material	biotic	above
1	Species richness Diplopoda	Above Decomposer Diversity	50	static	decomposer	carbon	excluded	information	biotic	above
2	Species richness saprophagous Diptera	Above Decomposer Diversity	50	static	decomposer	carbon	excluded	information	biotic	above
4	Microbial biomass* (substrate induced respiration)	Below Decomposer Abundance	82	static	decomposer	carbon	Carbon	material	biotic	below
2	Number of Collembola	Below Decomposer Abundance	82	static	decomposer	carbon	excluded	material	biotic	below
1	Number of Enchytraeidae	Below Decomposer Abundance	82	static	decomposer	carbon	excluded	material	biotic	below

4	Number of endogeic earthworms	Below Decomposer Abundance	82	static	decomposer	carbon	excluded	material	biotic	below
3	Number of Isopoda	Below Decomposer Abundance	82	static	decomposer	carbon	excluded	material	biotic	below
2	Number of *Lumbricus terrestris*	Below Decomposer Abundance	82	static	decomposer	carbon	excluded	material	biotic	below
2	Number of Oribatid mites	Below Decomposer Abundance	82	static	decomposer	carbon	excluded	material	biotic	below
4	Soil basal respiration	Below Decomposer Abundance	82	static	decomposer	carbon	carbon	material	biotic	below
1	Microbial C%	Microbial C%	77	static	ecosystem	carbon	carbon	material	biotic	below
1	Number of Arachnida	Above Carnivore Abundance	50	static	carnivores	carbon	excluded	material	biotic	above
1	Hymenoptera parasitited broad cells	Above Carnivore Abundance	76	static	carnivores	carbon	excluded	material	biotic	above
1	Number of Arachnida Vegetation	Above Carnivore Abundance	50	static	carnivores	carbon	excluded	material	biotic	above
1	Number of Arachnida ground	Above Carnivore Abundance	50	static	carnivores	carbon	excluded	material	biotic	above
2	Number of Carabidae	Above Carnivore Abundance	50	static	carnivores	carbon	excluded	material	biotic	above
5	Number of Chilopoda	Above Carnivore Abundance	50	static	carnivores	carbon	excluded	material	biotic	above
2	Number of parasitic Hymenoptera	Above Carnivore Abundance	50	static	carnivores	carbon	excluded	material	biotic	above
1	Number of parasitic Diptera	Above Carnivore Abundance	50	static	carnivores	carbon	excluded	material	biotic	above
1	Number of Predatory Heteroptera	Above Carnivore Abundance	50	static	carnivores	carbon	excluded	material	biotic	above
1	Number of Predatory Hymenoptera	Above Carnivore Abundance	50	static	carnivores	carbon	excluded	material	biotic	above
1	Number of predatory Diptera	Above Carnivore Abundance	50	static	carnivores	carbon	excluded	material	biotic	above
2	Number of predatory Coleoptera	Above Carnivore Abundance	82	static	carnivores	carbon	excluded	material	biotic	above
3	Number of Araneae	Above Carnivore Abundance	82	static	carnivores	carbon	excluded	material	biotic	above
2	Number of Staphylinidae	Above Carnivore Abundance	50	static	carnivores	carbon	excluded	material	biotic	above
1	Hymenoptera species number parasites	Above Carnivore Diversity	76	static	carnivores	carbon	excluded	information	biotic	above
1	Species richness Arachnida Vegetation	Above Carnivore Diversity	50	static	carnivores	carbon	excluded	information	biotic	above
2	Species richness Arachnida ground	Above Carnivore Diversity	50	static	carnivores	carbon	excluded	information	biotic	above

2	Species richness Carabidae	Above Carnivore Diversity	50	static	carnivores	carbon	excluded	information	biotic	above
1	Species richness Chilopoda	Above Carnivore Diversity	50	static	carnivores	carbon	excluded	information	biotic	above
2	Species richness Staphylinidae	Above Carnivore Diversity	50	static	carnivores	carbon	excluded	information	biotic	above
1	Species richness zoophagous Diptera	Above Carnivore Diversity	50	static	carnivores	carbon	excluded	information	biotic	above
1	Species richness zoophagous heteroptera	Above Carnivore Diversity	50	static	carnivores	carbon	excluded	information	biotic	above
2	Species richness zoophagous Hymenoptera	Above Carnivore Diversity	50	static	carnivores	carbon	excluded	information	biotic	above
1	Number of bacteria feeding nematodes	Below Carnivore Abundance	73	static	carnivores	carbon	excluded	material	biotic	below
2	Number of Gamasida	Below Carnivore Abundance	82	static	carnivores	carbon	excluded	material	biotic	below
1	Number of nematode feeding nematodes	Below Carnivore Abundance	73	static	carnivores	carbon	excluded	material	biotic	below
1	Number of predatory nematodes	Below Carnivore Abundance	73	static	carnivores	carbon	excluded	material	biotic	below
2	Number of predatory Coleoptera larvae	Below Carnivore Abundance	82	static	carnivores	carbon	excluded	material	biotic	below
6	Soil CO_2 emission rate	Soil CO_2 emission	79	dynamic	ecosystem	carbon	carbon	material	abiotic	below
6	$\delta^{13}C$ soil†	$\delta^{13}C$ soil	82	dynamic	ecosystem	carbon	carbon	material	abiotic	below
6	Soil methane oxidation	Soil methane oxidation	79	dynamic	ecosystem	carbon	carbon	material	abiotic	below
12	Storage soil C inorganic%†	Storage soil C inorganic%	82	dynamic	ecosystem	carbon	carbon	material	abiotic	below
12	Storage soil C organic%†	Storage soil C organic%	82	dynamic	ecosystem	carbon	carbon	material	abiotic	below
4	Plant $\delta^{15}N$	Plant $\delta^{15}N$	81	static	producer	nutrient	nutrient	material	biotic	above
11	Plant N%	Plant N%	79	static	producer	nutrient	nutrient	material	biotic	above
16	Ammonium soil%	Ammonium soil%	82	static	ecosystem	nutrient	nutrient	material	abiotic	below
2	Denitrifying enzyme activity	Microbial N	80	dynamic	ecosystem	nutrient	nutrient	material	biotic	below
1	Microbial N%	Microbial N	77	static	ecosystem	nutrient	nutrient	material	biotic	below
2	Nitrifying enzyme activity	Microbial N	79	dynamic	ecosystem	nutrient	nutrient	material	biotic	below
6	Soil $\delta^{15}N$†	Soil $\delta^{15}N$	82	dynamic	ecosystem	nutrient	nutrient	material	abiotic	below
6	Soil N_2O emission	Soil N_2O emission	79	dynamic	ecosystem	nutrient	nutrient	material	abiotic	below
2	Fine root N%†	Root N%	75	dynamic	producer	nutrient	nutrient	material	biotic	below
12	Storage soil N%†	Storage soil N%	82	dynamic	ecosystem	nutrient	nutrient	material	abiotic	below

19	Soil nitrate%	Soil Nitrate%	82	static	ecosystem	nutrient	nutrient	material	abiotic	below
2	Soil P%	Soil P%	82	static	ecosystem	nutrient	nutrient	material	abiotic	below
1	Gravimetric water content %	Soil Water	82	static	ecosystem	water	water	material	abiotic	below
37	Soil water content	Soil Water	82	static	ecosystem	water	water	material	abiotic	below
3	Plant $\delta^{13}C$‡	Plant $\delta^{13}C$	82	static	producer	carbon	excluded	material	biotic	above

* Microbial biomass was measured as substrate induced respiration and is therefore a direct measure of carbon ($C0_2$ production). Substrate induce respiration is commonly used as a measure of microbial carbon and has been shown to be highly correlated with microbial carbon content analysed using other methods (Beck et al. 1997).

‡ Plant $\delta^{13}C$ values were analysed as carbon variables because they have been shown to be associated with a light adapting strategy (Roscher et al. 2011). We excluded them from the analysis of variables directly associated with the different biogeochemical cycles because Plant $\delta^{13}C$ variables are also associated with water use efficiency (Farquhar et al. 1982) and therefore are associated with both the water and carbon cycles. Repeating the full analysis with Plant $\delta^{13}C$ assigned to the water cycle does not alter the significance of the biogeochemical cycle term ($p=0.01$ on addition to the null model and $p=0.03$ on deletion from the full model; see methods).

† These variables were calculated as differences between pairs of years and are therefore considered dynamic.

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Chapter 3

Bryophyte community assembly along a vascular plant biodiversity gradient.

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Abstract

A number of grassland biodiversity experiments have explored the relationship between biodiversity and ecosystem processes. Within one such research platform in Germany (Jena Experiment) we recorded the composition of the bryophyte community in 2006 and 2008. Vascular plant communities were sown on a former arable field in 2002 with a gradient of species richness (1, 2, 4, 8, 16, and 60 species). Across all communities and both years a total of 29 moss species were recorded. Bryophyte species richness responded negatively to increasing vascular plant species richness mostly as a result of the increased cover of the vascular plant community. In 2006 communities initially sown in monoculture had on average 5.3 (±0.61) bryophyte species per plot whereas in communities originally sown with 60 species there were on average 1.5 (±0.65) species per plot. By 2008 this range had contracted. Beyond the biodiversity gradient, in bare ground plots bryophyte richness was comparable to monocultures in both years. Comparison of succession plots (3.5±1.5 species per plot) and neighboring control meadows (8.5±0.5 species per plot) suggests dispersal limitation which may be an important consideration for meadow restoration. Principal Coordinate's (PCO) analysis of the presence/absence (2006/2008) and abundance (2008) of bryophyte species revealed good differentiation of bryophyte species composition along the vascular plant species richness gradient. The majority of the differences were attributable to the presence of grasses and legumes in a community. Similarly, bryophyte richness and cover responded positively to grass presence, and negatively to legume presence. Grass species might facilitate bryophytes by optimising abiotic conditions for bryophyte establishment and growth, whereas legumes may have the opposite effect, or cause toxic fertilisation of bryophytes. Not all components of a plant community respond positively to increased vascular species richness, advocating increased richness of one taxonomic group over another in grassland may reduce total community diversity.

Introduction

Components of vascular plant biodiversity have been shown to influence various ecosystem functions (Balvanera et al. 2006, Hector et al. 1999, Scherber et al. 2010, Allan et al. 2013). However plant communities are not limited to vascular plant life, and

the interactions within and between different life forms in a community will influence both community composition and properties of ecosystem functioning. One method for exploring the interactions between different life forms is to study community assembly processes (Götzenberger et al. 2011, Weiher et al. 2011). By focusing on how non-vascular communities assemble in response to vascular plants we gain insight into the direction and intensity of these interactions which determine plant community structure. By including a gradient of vascular plant biodiversity we can also examine how much the intensity of these interactions is subject to how many species are present in a system (MacArthur 1972, Levine 1999). In this study we primarily explore how two components of vascular plant biodiversity, namely species richness and functional group richness, influence the assembly of bryophyte communities.

Bryophyte physiology

Bryophytes and vascular plants share much of a common physiology (Proctor 2000a). Where bryophytes do differ, such dissimilarities relate more to the physical practicalities of small size than to evolutionary primitiveness (Proctor 2000a, Proctor 2000b, Ejrnæs & Poulsen 2001). One key difference between bryophytes and vascular plants is their adaptation to the intermittent availability of water (Proctor 2000a). Where vascular plants use xylem to transport water from soil to leaves and shoots, most bryophytes have evolved a poikilohydric habit permitting desiccation tolerance (Green & Lang 1995, Proctor 2000b). When moisture is readily available, bryophytes photosynthesize and grow, when drought occurs, bryophytes suspend their metabolism (Proctor 2000b, Proctor 2000c, Pederson et al. 2001). But this split is hazy and various degrees of internal structuring for water transport can be found in bryophyte species.

Many bryophyte species are ectohydric, water conduction is almost entirely external (van Tooren et al. 1990, Pharo et al. 1999, Proctor 2000a, Peintinger & Bergamini 2006). Ectohydric species attain nutrients by intercepting and absorbing solutes in rainwater, mist droplets, throughfall precipitation and by dry deposition of airborne dust and gases (van Tooren et al. 1990, Bates 2000, Proctor 2000a). Other species are endohydric, water and nutrients are transported from the base of the plant via a well-developed central grouping of specialised water-conducting cells, making

substrate an important resource (Proctor 2000a, Frey et al. 2006). Mixohydric species represent a balance of the two types, relying on a combination of both internal and external transport systems (Proctor 2000a). In general bryophytes are separated into what are essentially two functional groups, acrocarpous and pleurocarpous species, based on the orientation and branching of their stems, and the position of their sex organs (archegonia) (Buck & Goffinet 2000, Frey et al. 2006). There is not a definitive split between acrocarpous and pleurocarpous species in terms of their predominant water and nutrient uptake process, but most pleurocarpous species are ectohydric and the majority of acrocarpous species are at least partially endohydric (Richardson 1981, Bates 2000).

A final point of difference between bryophytes and vascular plants is dispersal. Bryophyte species are both spore-dispersed and reproduce vegetatively from tiny fragments of the gametophyte, there is no difference in the success of either propagation system, and both processes together allow bryophytes to disperse easily across landscapes (Pharo et al. 1999, Shaw 2000, Tan & Pócs 2000, Frahm 2008).

Bryophytes in grassland

Bryophytes occur in a great variety of habitats, and can represent a major component of the plant community in terms of primary production and species richness (During & van Tooren 1987, Bergamini et al. 2001). However, in temperate zones and in grasslands bryophytes are generally less prominent. In grassland systems bryophytes often form a distinct layer under the vascular plant community (Ingerpuu et al. 2005). Biomass production and species richness of bryophytes in grasslands has been shown to be closely tied to the productivity of the system, in highly productive systems both metrics are reduced whereas in increasingly oligotrophic systems both measures can be substantially higher (Virtanen et al. 2000, Aude and Ejrnæs 2005, Hejcman et al. 2010). Bryophytes in grasslands are typically inconspicuous in the dry summer months, bryophyte growth and peak abundance generally takes place during the moist and cool seasons from autumn through spring (van Tooren et al. 1990, Virtanen et al. 2000). In recent years concerns over the loss of bryophyte species richness in grasslands as a

result of changing agricultural practices have been raised (Ejrnæs & Poulsen 2001a, Peintinger & Bergamini 2006).

Bryophyte interactions

Bryophytes are involved in a complex variety of interactions with many taxonomic groups – competitive, parasitic, symbiotic and mutualistic relationships have been reported with vascular plants, algae, fungi, lichens, cyanobacteria and both autotrophic and heterotrophic bacteria (During & van Tooren 1990). In temperate grassland communities it is the interaction between bryophytes and vascular plants that is expected to have the greatest impact (Virtanen et al. 2000, Zamfir et al. 2000, Aude and Ejrnæs 2005, Ingerpuu et al. 2005). Many studies have explored the impact that established bryophyte communities have on germination and early life history stages of vascular plants, reporting reduced seed germination and suppressed seedling establishment (Keizer et al. 1985, During & van Tooren 1987, Kotorová and Lepš 1999, Zamfir 2000, Ingerpuu et al. 2003, Ingerpuu & Kupper 2007, Jeschke & Kiehl 2008, Gornall et al. 2011, Soudzilovskaia 2011). There is a general acceptance that bryophytes have almost no biotic effect on mature vascular plants.

Fewer studies have examined how vascular plant species influence bryophytes (Ingerpuu et al. 2003, Ingerpuu et al. 2005). Negative relationships between vascular plant cover and bryophyte species richness in grasslands suggests that bryophytes are poor competitors in these systems (Watson 1960, van Tooren et al. 1988, During & van Tooren 1990, Virtanen et al. 2000, Ingerpuu et al. 2005, Peintinger & Bergamini 2006). In general, increases in vascular plant biomass have resulted in a decrease in bryophyte biomass (Virtanen et al. 2000, Aude and Ejrnæs 2005, Hejcman et al. 2010). While the opposite has also been demonstrated, increasing vascular plant biomass can facilitate bryophytes by increasing ambient moisture in the bryophyte layer (Ingerpuu et al. 2005, Hejcman et al. 2010).

Particular functional groups of vascular plants might have specific impacts on bryophyte communities. The presence of legumes in grasslands has been shown to increase the availability of nitrogen in soil (Oelmann 2007). Plant communities dominated by legumes might be expected to harbour a bryophyte community dominated

by acrocarpous species that can exploit increased nitrogen availability. Acrocarpous bryophytes are common and diverse in disturbed habitats, and are typical of systems with a history of arable use (Cornish 1954). Alternatively, communities dominated by grass species, without a nitrogen supply, could be dominated by pleurocarpous bryophyte species — that rely more on dry and wet deposition as a source of nutrients (Richardson 1981, Bates 2000). Pleurocarpous species are also slower colonisers, therefore reduced bryophyte richness could be expected in communities dominated by grasses (Cornish 1954, Gimingham & Birse 1957, Watson 1960, Richardson 1981, Bates 2000).

In an experimental grassland (Jena Experiment) with a gradient of species richness (1, 2, 4, 8, 16, and 60 species) and functional group richness (1-4 functional groups) we investigated the relationship between vascular plant biodiversity metrics and bryophyte species. We surveyed bryophyte species richness and community composition in 2006 and 2008. The two survey windows, respectively 5 and 7 years after the experimental platform was established, permitted examination of ongoing colonisation processes.

Hypotheses

1. Bryophyte species richness will decrease with increasing vascular plant biodiversity (species- and functional group richness)
2. This decrease will be explained by increasing vascular plant cover
3. Increasing vascular plant biodiversity increases micro-environmental heterogeneity and hence variance in bryophyte species richness
4. Bryophyte species richness and the proportion of acrocarpous species will decrease with increasing proportions of grass species
5. Bryophyte species richness and the proportion of acrocarpous species will increase with increasing proportions of legume species
6. Composition of bryophyte communities will fluctuate in response to the composition of vascular plant communities

Methods

Experimental setup

The present study was carried out in the Jena Experiment (Germany), a large experimental platform designed to examine the effects of grassland biodiversity on ecosystem functioning (Roscher *et al.* 2004). The experimental site is located on the floodplain of the Saale River near the city of Jena (Thuringia, Germany, 50°55' N, 11°35' E, 130 m a.s.l.). The mean annual air temperature is 9.3 °C, and the mean annual precipitation is 587 mm (Kluge and Müller-Westermeier 2000). The soil is a nutrient-rich Eutric Fluvisol developed from up to 2m-thick loamy fluvial sediments. Until the establishment of the biodiversity experiment the land was used for arable crops having been converted from grassland in the 1960s. The field was ploughed and kept fallow in 2001, and after being harrowed repeatedly, experimental grassland communities were sown in May 2002. Seventy-eight experimental plots were established with randomly assembled communities of 1, 2, 4, 8, or 16 species. The Jena Experiment species pool consisted of 60 native central European plant species chosen to resemble semi-natural species-rich mesophilic grassland, akin to a Molinio-Arrhenatheretea meadow (Ellenberg 1988). The 60 species were categorized into four functional groups derived from a cluster analysis of ecological and morphological traits, the groups were 16 grasses, 12 legumes, 12 small forbs, and 20 tall forbs. The number of functional groups (richness of functional groups) was varied as much as possible within the species richness levels to achieve an almost orthogonal design with respect to functional-group composition and species richness (Roscher *et al.* 2004). Communities were established in 20x20 m plots that were arranged in four blocks each with the same number of plots per level of species richness and plant functional group number. Each block also contained a bare ground plot (without vascular plants) and a plot sown with a mixture of the complete 60 species pool. The sown species combinations were maintained by weeding twice per year (April, July). Herbicides were used as spot-treatments against some weeds (*Cirsium arvense* (L.) Scop., *Rumex* spec.), and where sown species combinations allowed for their application (bare ground plots, against herbs in pure grass communities and against grasses in pure herb communities, respectively). In addition, two succession plots (without sowing a seed

mixture and alowing for spontaneous colonisation of vascular plants) were established. Plots were mown bi-annually and no fertiliser was applied. Two control plots were installed in adjacent old semi-natural meadows.

In each 20x20 m plot a 2x2 m subplot was identified and subdivided into a grid of 10x10 cm sub-squares for sampling of bryophytes in early December 2006 and November 2008. Five sub-squares were selected using random numbers. The sub-squares that were randomly located in 2006 were not visited again in 2008, but a new complement of randomly located sub-squares was selected. In each sub-square the per cent cover and species richness of vascular plant species was recorded in November/December. The complete bryophyte cover in each sub-square was collected for identification and quantification. In 2008 the per cent cover of moss species was also recorded. All bryophyte species were identified according to Frahm & Frey 1992, Nebel & Philippi 2000, Nebel & Philippi 2001. For the 2008 data abundances were calculated based on the measures of moss cover per subsample. In some sub-squares determination of bryophytes to species level was not possible because sporophyte material was not present. The outcome of this is the production of three species pairs, where the identified material is one of the two species in the pair; these are *Funaria hygrometrica* Hedw. - *Physcomitrium pyriforme* (Hedw.) Brid., *Didymodon* species - *Pseudocrossidium* species, and *Weissia brachycarpa* (Nees & Hornsch.) Jur. - *Weissia controversa* Hedw. For each pair only the first species in the pair is referred to from here forward in the paper. Two species in the genus *Dicranella* were identified; *Dicranella varia* (Hedw.) Schimp. and *Dicranella staphylina* H. Whitehouse, where it is not possible to differentiate between the two the sample has been designated *Dicranella* species. For several other samples it was not possible to determine *Pottia*, *Weissia* or *Bryum* to the species-level because the sporophyte was not sufficiently developed, in these situations the epithet species has been used. A complete list of bryophyte species is available in appendix A.

Statistical analysis

Bryophyte species richness and composition were summed from the 5 sub-squares measured in each plot, and the cover was averaged. The CV of each metric was calculated. All analyses were completed as linear models in R (version 2.13). Bryophyte species richness was analysed against block and the two experimental factors sown vascular plant species richness and sown vascular plant functional group richness. The contrast for bare ground was fit before sown vascular plant species richness, and when the dependent variable was measured both in 2006 and 2008, year was fit before bare ground, as well as the interactions between year and sown vascular plant species richness and year and sown vascular plant functional group richness. Models were run with and without vascular plant cover as covariate, which when included was fit between bare ground and sown vascular plant species richness. Functional groups of vascular plants were included in the analyses in two ways. Firstly, separate models including the sown relative proportion of each functional group fit after the experimental factors were run. Secondly, separate models were run including the contrast of functional group presence. Both types of models were run with and without vascular plant cover being included before sown vascular plant species richness. An additional metric related to bryophyte species richness was analysed, the proportion of acrocarpous species in a community, the number of acrocarpous divided by total bryophyte richness. The proportion of acrocarpous species was analysed in the same manner as is described above for bryophyte species richness.

Similarly, the relationship between total bryophyte cover which was measured in 2008 was analysed in the same manner as above but without the inclusion of year. Individual bryophyte species that occurred in more than 10 plots were analysed separately. These analyses were completed for both 2006 and 2008. Total cover of the bryophyte community was not measured in 2006, therefore presence/absence of individual species was analysed for 2006. Analyses were completed in the same manner as for bryophyte species richness but without the inclusion of year. The abundance of individual bryophyte species that occurred in more than 10 plots was analysed for 2008. Given the difficulty with field identifications of bryophyte species, the

abundance per sub-square in 2008 was derived from the total bryophyte cover in each sub-square and the proportion of each species collected in the sample.

Analyses of the bryophyte community were completed using Principal Coordinates (PCO) analyses, which were performed for bryophyte presence/absence in 2006 and 2008 using a Jaccard similarity index, and separately on the abundance of moss species in 2008 using Bray-Curtis distances . All PCO analyses were completed in Canoco. Analysis of Variance (ANOVA) of each principal component was completed with samples scores. Mantel correlations were calculated between bryophytes in 2006 and 2008, bryophytes in 2008, sown vascular plants, and cover of litter and sown species.

Results

Bryophyte species richness

Bryophyte species richness varied significantly between blocks (table 1, F = 6.4831, P = 0.0006) but there was not a gradient in bryophyte species richness across the plots. Bryophyte species richness in communities belonging to the sown vascular plant species richness gradient increased on average from 3.9 (±0.32) species per plot in 2006 to 5.6 (±0.23) species per plot in 2008. Year was one of the most important factors influencing bryophyte species richness (table 1, F = 28.1319, P = <0.0001).

Table 1. ANOVA output of linear model with bryophyte species richness as the dependent variable and the experimental factors and time as explanatory variables. Explanation of abbreviated names in table: Df (degrees of freedom), SS (sums of squares), MS (mean squares), F (F value), P (P value), SVP (sown vascular plant). Significance codes for P value: 0.001 = ***, 0.01 = **, 0.05 = *.

effect	Df	SS	MS	*F*	P	S
block	*3*	*73.26*	*24.42*	*6.4831*	*0.0006*	**
bare ground	1	7.81	7.81	2.0725	0.1541	
SVP species richness	*5*	*119.89*	*23.98*	*6.3663*	*0.0001*	***
SVP functional group richness	4	31.30	7.82	2.0772	0.0922	.
year	*1*	*105.96*	*105.96*	*28.1319*	*<0.0001*	***
plot	*72*	*525.13*	*7.29*	*1.9364*	*0.0025*	**
year x SVP species richness	6	16.17	2.70	0.7157	0.6381	
year x SVP functional group richness	4	5.88	1.47	0.3901	0.8151	
residuals	75	282.49	3.77			

Table 2. ANOVA output of linear model with bryophyte species richness as the dependent variable and vascular plant per cent cover and the experimental factors and time as explanatory variables. Explanation of abbreviated names in table: Df (degrees of freedom), SS (sums of squares), MS (mean squares), F (F value), P (P value), SVP (sown vascular plant). Significance codes for P value: 0.001 = ***, 0.01 = **, 0.05 = *.

effect	Df	SS	MS	*F*	P	S
block	*3*	*73.26*	*24.42*	*6.4074*	*0.0006*	***
bare ground	1	7.81	7.81	2.0484	0.1566	
SVP cover	*1*	*171.22*	*171.22*	*44.9283*	*<0.0001*	***
SVP species richness	5	41.70	8.34	2.1882	0.0645	.
SVP functional group richness	4	32.38	8.10	2.1244	0.0862	.
year	*1*	*28.02*	*28.02*	*7.3516*	*0.0083*	**
plot	*72*	*513.56*	*7.13*	*1.8716*	*0.0040*	**
year x SVP species richness	6	12.01	2.00	0.5253	0.7874	
year x SVP functional group richness	4	5.92	1.48	0.3882	0.8165	
residuals	74	282.01	3.81			

The two experimental metrics of biodiversity were fitted in models with and without vascular plant per cent cover (table 1 and 2). Sown vascular plant species richness had a large effect on bryophyte species richness (table 1, $F = 6.3663$, $P = 0.0001$), on average bryophyte species richness decreased with increasing sown vascular plant species richness (figure 1). This effect was largely caused by sown vascular plant species cover, which explained most of the variation attributable to sown vascular plant species richness (table 2, for SVP cover $F = 44.9283$, $P = <0.0001$). An analysis of the coefficient of variation (CV) of bryophyte species richness revealed variation in richness to increase significantly with increasing sown vascular plant species cover (figure 2, table B1, $F = 23.2656$, $P = <0.0001$).

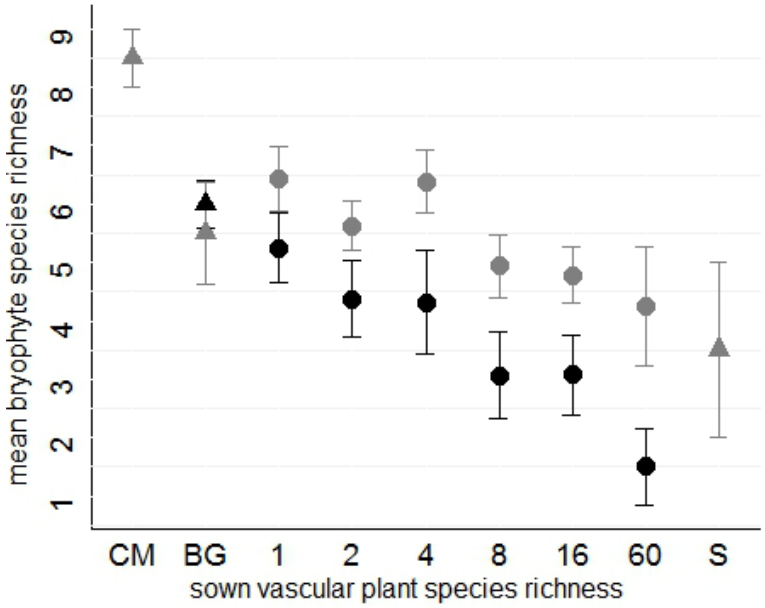

Figure 1. Mean and the standard error of bryophyte species richness per plot in 2006 in black ( ) and 2008 in grey ( ). Circular points ( ) correspond to plots that comprise a level of the vascular plant species richness gradient, triangular points ( ) correspond to plots that do not comprise a level of the vascular plant species richness gradient. Statistical analyses included only those points in the vascular plant species richness gradient in addition to the bareground (BG) plots. CM denotes control meadow plots, S denotes mown succession plots.

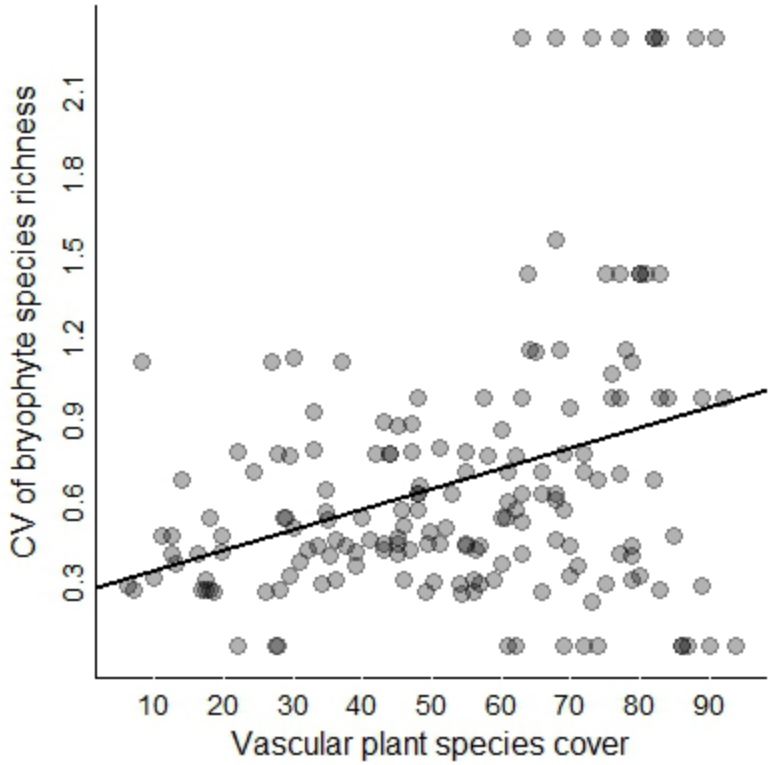

Figure 2. Coefficient of variation (CV) of bryophyte species richness against vascular plant species cover.

In 2006 communities initially sown in monoculture had on average 5.3 (±0.61) bryophyte species per plot whereas on the opposite end of the species richness gradient, communities originally sown with 60 species on average 1.5 (±0.65) bryophyte species per plot. By 2008 this range had contracted, and on average 6.4 (±0.56) bryophyte species per plot occurred in communities sown in monoculture versus 4.3 (±1.03) bryophyte species per plot in communities initially sown with 60 species.

Separate models were run for the sown relative proportions of each vascular plant functional group, both with and without fitting sown vascular plant species cover before the experimental variables. Also models including the contrast between the presence and absence of each functional group in each community were run, both with and without fitting sown vascular plant species cover before the experimental variables. The sown relative proportion of legume species significantly influenced bryophyte species richness (table B2, $F = 5.4390$, $P = 0.0224$), however the majority of this variation was explained by vascular plant species cover (table B3). The contrast of grass presence or absence in a community had a significant impact on bryophyte

species richness (table B4, $F = 8.8868$, $P = 0.0039$). Bryophyte species richness was higher in plots with grasses in 2006, and higher in plots without grasses in 2008 (figure 3). The interaction between year and grass contrast was significant (table B4, $F = 8.4231$, $P = 0.0049$) and vascular plant cover explained little of this variation (table B5). Bryophyte species richness was much lower in plots with legumes in 2006 and still somewhat lower in 2008 (figure 3, table B6, $F = 9.2693$, $P = 0.0039$). Vascular plant cover also explained little of this variation (table B7).

Beyond the targeted biodiversity gradient, in bare ground plots bryophyte richness was comparable to monocultures in 2006 (6±0.41 species per plot), and slightly lower in 2008 (5.5 ±0.87 species per plot) (figure 3). The neighboring control meadows and the mown succession communities permit insight into both dispersal processes and disturbance in the experiment. Both of these community types were measured in 2008, bryophyte species richness was on average 3.5 (±1.5) species per plot in the mown succession communities and much higher in the neighboring control meadows where an average of 8.5 (±0.5) species per plot were identified.

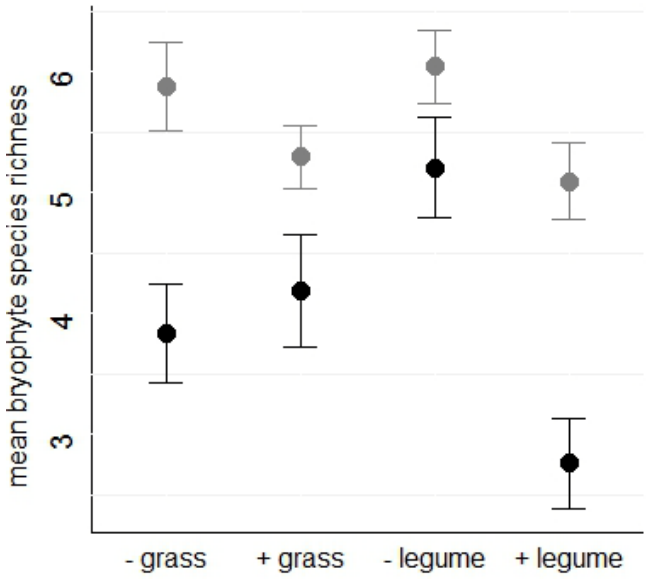

Figure 3. Mean and the standard error of bryophyte species richness per plot in 2006 in black ( ) and 2008 in grey ( ). Statistical analyses included only those points in the vascular plant species richness gradient in addition to the bareground (BG) plots. CM denotes control meadow plots, S denotes mown succession plots.

An additional analysis of the proportion of acrocarpic bryophyte species was attempted. The contrast between initially bare ground and not bare ground plots was significant in this analysis (table 3, F = 5.5636, P = 0.0210). In 2006 on average 0.92 (±0.04) of the bryophyte species were acrocarpous versus 0.74 (±0.08) in the remainder of plots (figure B1). In 2008 all bryophyte species in bare ground plots were acrocarpous versus 0.76 (±0.03) of the remaining plots. With increasing vascular plant cover the proportion of acrocarpous species significantly decreased (coefficient = -0.006 in 2006, coefficient = -0.004 in 2008, table 3, F = 16.8655, P = 0.0001). With increasing sown vascular plant species richness the proportion of acrocarpic bryophyte species significantly decreased in both 2006 and 2008 (figure B1, table 3, F = 5.0177, P = 0.0005). Both the relative proportion of grass species (table B8, F = 7.0717, P = 0.0096) and the contrast of grass presence significantly impacted on the proportion of acrocarpic bryophyte species (table B9, F = 9.5992, P = 0.0028).

Table 3. ANOVA output of linear model with proportion of acrocarpic bryophyte species as the dependent variable and vascular plant per cent cover and the experimental factors and time as explanatory variables. Explanation of abbreviated names in table: Df (degrees of freedom), SS (sums of squares), MS (mean squares), F (F value), P (P value), SVP (sown vascular plant). Significance codes for P value: 0.001 = ***, 0.01 = **, 0.05 = *.

effect	Df	SS	MS	F	P	S
block	*3*	*0.64*	*0.21*	*3.4762*	*0.0202*	*
bare ground	*1*	*0.34*	*0.34*	*5.5636*	*0.0210*	*
SVP cover	*1*	*1.03*	*1.03*	*16.8655*	*0.0001*	***
SVP species richness	*5*	*1.53*	*0.31*	*5.0177*	*0.0005*	***
SVP functional group richness	4	0.07	0.02	0.2804	0.8898	
year	1	0.05	0.05	0.7410	0.3921	
plot	*72*	*7.15*	*0.10*	*1.6289*	*0.0192*	*
year x SVP species richness	6	0.53	0.09	1.4572	0.2048	
year x SVP functional group richness	4	0.13	0.03	0.5154	0.7246	
residuals	74	4.51	0.06			

Bryophyte cover

Bryophyte cover was measured in 2008 and was significantly influenced by grass and legume functional groups. The average bryophyte cover was significantly higher in plots containing higher relative proportions of sown grass species (table B10, F = 2.8276, P = 0.0166). The contrast for grass presence in a community explained a large amount of the variation in bryophyte cover (table 4, F = 14.8178, P = 0.0003) which on average in communities sown with grass species was double that in communities without (figure 3). The contrast for legume presence in a community also explained some of the variation in bryophyte cover (table 5, F = 4.0453, P = 0.0482) which was lower in communities sown with legume (figure 4). Bryophyte cover could be seen to increase with sown vascular plant species richness, up to a threshold of species richness (figure 4). Mean bryophyte cover consistently increased from communities originally sown as monocultures of vascular plants up until communities sown with eight species of vascular plants (figure 5). In communities originally sown with more than eight vascular plant species the cover of bryophytes decreased, indicating a threshold at which vascular plant species richness no longer facilitates bryophyte cover (figure 4). Due to the high variation in the cover data this trend is not significant. It should be noted that bryophyte cover in the control meadows was generally three times greater than in any of the experimental communities (figure 4).

Table 4. ANOVA output of linear model with mean cover of bryophyte species as the dependent variable and vascular plant per cent cover and the experimental factors and the contrast of grass species presence as explanatory variables. Explanation of abbreviated names in table: Df (degrees of freedom), SS (sums of squares), MS (mean squares), F (F value), P (P value), SVP (sown vascular plant). Significance codes for P value: 0.001 = ***, 0.01 = **, 0.05 = *.

effect	Df	SS	MS	F	P	S
block	*3*	*1889.90*	*630.00*	*2.2119*	*0.0943*	*.*
bare ground	1	3.30	3.30	0.0116	0.9144	
SVP cover	1	2.70	2.70	0.0094	0.9232	
SVP species richness	5	1615.70	323.10	1.1346	0.3503	
SVP functional group richness	4	1040.80	260.20	0.9136	0.4610	
grass contrast	*1*	*4220.20*	*4220.20*	*14.8178*	*0.0003*	*****
residuals	70	19936.50	284.80			

Table 5. ANOVA output of linear model with mean cover of bryophyte species as the dependent variable and vascular plant per cent cover and the experimental factors and the contrast of legume species presence as explanatory variables. Explanation of abbreviated names in table: Df (degrees of freedom), SS (sums of squares), MS (mean squares), F (F value), P (P value), SVP (sown vascular plant). Significance codes for P value: 0.001 = ***, 0.01 = **, 0.05 = *.

effect	Df	SS	MS	F	P	S
block	3	1889.90	629.98	1.9310	0.1325	
bare ground	1	3.30	3.31	0.0102	0.9200	
SVP cover	1	2.70	2.66	0.0082	0.9283	
SVP species richness	5	1615.70	323.14	0.9905	0.4299	
SVP functional group richness	4	1040.80	260.20	0.7976	0.5308	
legume contrast	*1*	*1319.70*	*1319.74*	*4.0453*	*0.0482*	*
residuals	70	22837.00	326.24			

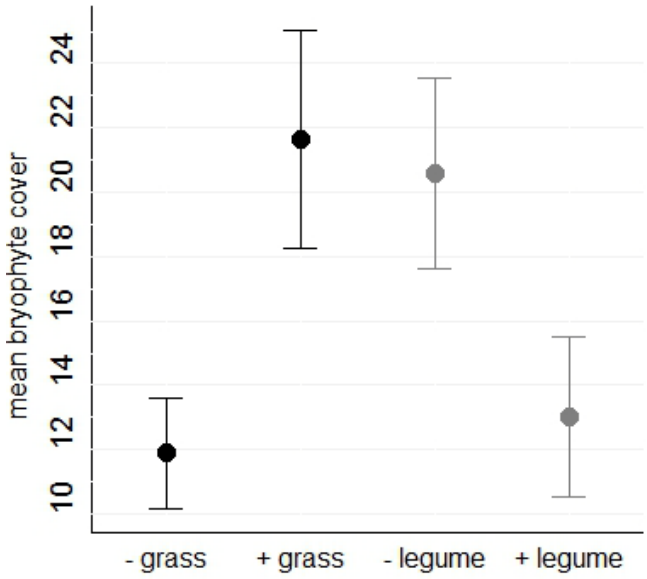

Figure 4. Mean and the standard error of bryophyte cover in plots with and without legume and grass species.

Individual bryophyte species response

Analyses were also completed for individual bryophyte species that occurred in more than 10 plots. The presence of individual bryophyte species was analysed for 2006 and the abundance of individual bryophyte species was analysed for 2008. For each species 5 analyses were completed, the first included only vascular plant cover and the

experimental factors. The remaining four analyses each included the contrast for presence of one of the four functional groups. In 2006 13 bryophyte species (including tag names) occurred in 10 or more plots. The responses of individual moss species were inconsistent; however significant trends were always in the same direction (table 6). The presence of legume species and increasing vascular plant cover caused species to occur less in plots. The presence of grass species and for two species the presence of tall herb species resulted in species occurring more in plots. The three species which respond positively to the presence of grass species — *Amblystegium serpens*, *Brachythecium rutabulum* and *Eurhynchium hians* —are the three pleurocarp species that occur in this group (table 6).

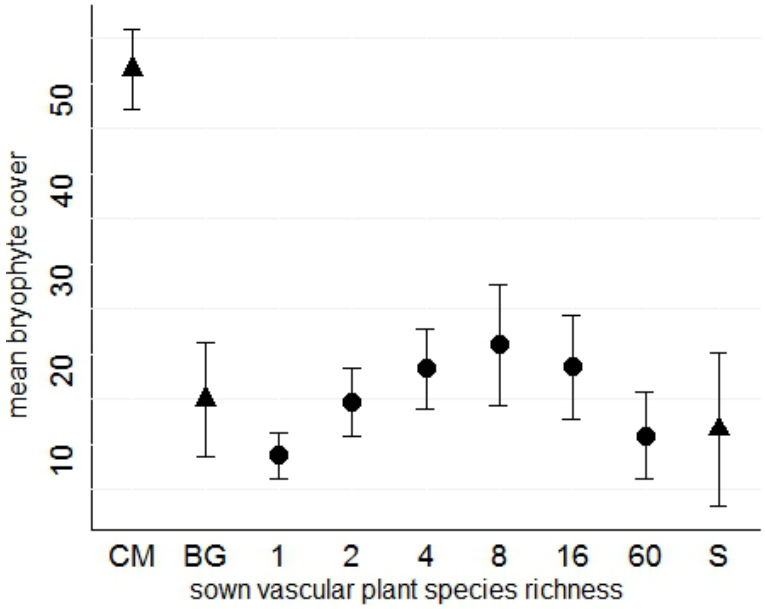

Figure 5. Mean and the standard error of bryophyte cover per plot. Circular points ( ) correspond to plots that comprise a level of the vascular plant species richness gradient, triangular points ( ) correspond to plots that do not comprise a level of the vascular plant species richness gradient. Statistical analyses included only those points in the vascular plant species richness gradient in addition to the bareground (BG) plots. CM denotes control meadow plots, S denotes mown succession plots.

In 2008 15 bryophyte species (including tag names) occurred in 10 or more plots. The responses of individual moss species were more idiosyncratic than 2006 (table 6). Once again the species which respond positively to the presence of grass species — *Amblystegium serpens* and *Brachythecium rutabulum* — are pleurocarp species (table 6). Fewer species respond negatively to the presence of legumes in 2008, and two acrocarpic species — *Bryum bicolor* and *Weissia longifolia* — respond positively to legume species. The increased presence of protonema in plots with legumes and the decreased presence in plots with grasses may be an indicator that these effects are important for growth of the gametophyte.

Table 6. Summary of significant effects from ANOVA outputs for five linear models for the presence of each bryophyte species that occurred in more than 10 plots in 2006. Explanation of abbreviated names in table: Df (degrees of freedom), SS (sums of squares), MS (mean squares), F (F value), P (P value), SVP (sown vascular plant). Significance codes for P value: 0.001 = ***, 0.01 = **, 0.05 = *.

species	n	block	BG	cover	SVPSR	SVPFGR	grass	legume	small	tall
Amblystegium serpens	11						↑*			
Barbula unguiculata	62			↓*				↓***		↑**
Brachythecium rutabulum	17						↑***	↓**		
Bryum bicolor	20		↑*	↓*						↑**
Bryum rubens	19	*		↓*						
Bryum sp.	10									
Dicranella staphylina	13					↓*		↓*		
Dicranella varia	11							↓*		
Dicranella sp	15									
Eurhynchium hians	31	**					↑***	↓*		
Phascum cuspidatum	47				↓*					
Weissia longifolia	32									
Weissia sp.	19									

Table 7. Summary of ANOVA outputs for five linear models for the abundance of each bryophyte species that occurred in more than 10 plots in 2008. Explanation of abbreviated names in table: Df (degrees of freedom), SS (sums of squares), MS (mean squares), F (F value), P (P value), SVP (sown vascular plant). Significance codes for P value: 0.001 = ***, 0.01 = **, 0.05 = *.

species	n	block	BG	cover	SVPSR	SVPFGR	grass	legume	small	tall
Amblystegium serpens	17						↑**			
Barbula unguiculata	71			↓***	↓*					
Brachythecium rutabulum	35						↑***	↓**		
Bryum bicolor	13	**	↑***				↓*	↑***		
Bryum rubens	20									
Bryum sp.	21									
Dicranella staphylina	21	*				**				
Dicranella varia	15				↓*					
Dicranella sp	14								↑*	
Eurhynchium hians	47			↑**						
Phascum cuspidatum	59									
Pottia davalliana	18	***		↓**		*				
Weissia longifolia	27							↑*		
Weissia sp.	19	*								
Protonema	35						↓**	↑*		

Principal coordinate analysis and mantel correlations

Analysis of the bryophyte community using PCO was completed for three community metrics, presence/absence in 2006 and 2008 (figure 6), and abundance in 2008 (figure 7). The majority of the variation in PCO 1 for the presence/absence of bryophytes in 2006 was explained by the presence of grass species in a community (table 8). The presence of tall herb species also explained some of this variation. The variation in PCO 2 was explained by sown vascular plant species richness, functional group richness and the presence of grass species. PCO 1 plotted against PCO 2 revealed good separation of sown vascular plant species richness levels (figure 6a). The majority of the variation in PCO 1 for the presence/absence of bryophytes in 2008 was explained by the presence of grass and legume species in a community (table 9). Sown vascular plant species richness and the bare ground contrast explained the majority of the remaining variation in PCO 1 (table 9). The variation in PCO 2 was explained by sown vascular plant species richness and the presence of legume species (table 9). PCO 1 plotted against PCO 2 again revealed good separation of sown vascular plant species richness

levels (figure 6b). The majority of the variation in PCO 1 for the abundance of bryophytes in 2008 was explained by the same effects as for bryophyte presence/absence, namely the presence of grass and legume species in a community, sown vascular plant species richness and the bare ground contrast (table 9). However where the presence of grass species has explained more variation for the presence/absence of bryophytes, both functional groups explained similar amounts of the variation in bryophyte abundance. The presence of grass species, legume species, and sown vascular plant species richness explained roughly equal amounts of the variation in PCO 2. PCO 1 plotted against PCO 2 revealed a clearer separation of sown vascular plant species richness levels than seen with presence/absence data (figure 7).

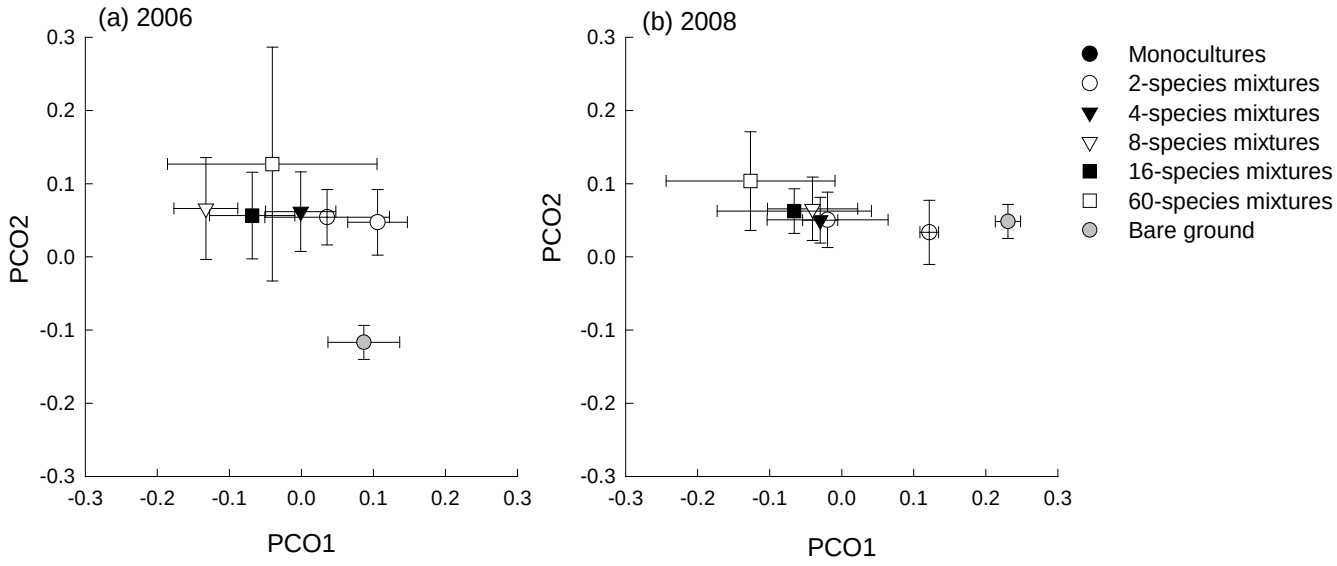

Figure 6. Principal Coordinate Analysis (PCO) - using Jaccard similarity based on bryophyte presence/absence in 2006 and 2008.

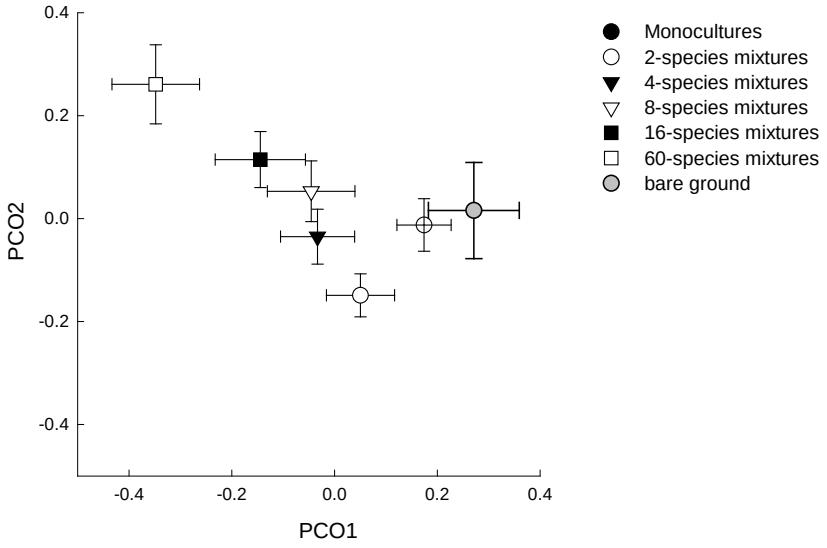

Figure 7. Principal Coordinate Analysis of - using Bray-Curtis distances based on moss abundances in 2008.

Table 8. Source of variation in each PCO for bryophyte species presence/absence in 2006. Each PCO is based on Jaccard similarity.

Source of variation		**PCO1**			**PCO2**			**PCO3**			**PCO4**		
	df	MS	F	p	MS	F	p	MS	F	p	MS	F	p
Block	3	0.250	6.22	0.001	0.052	1.49	0.224	0.029	0.80	0.498	0.024	1.27	0.289
Bare ground	1	0.029	0.72	0.397	0.060	1.74	0.192	0.007	0.21	0.652	0.503	**27.27**	**<0.001**
SR (log-linear)	1	0.157	3.93	0.051	0.298	**8.61**	**0.004**	0.001	0.02	0.883	0.021	1.12	0.293
FG (linear)	1	0.004	0.09	0.767	0.212	**6.13**	**0.016**	0.019	0.54	0.466	0.021	1.12	0.294
Legume (LE)	1	0.059	1.48	0.227	0.096	2.85	0.096	0.331	10.40	0.002	0.006	0.34	0.560
Grass (GR)	1	0.449	**13.05**	**0.001**	0.134	**4.03**	**0.048**	0.152	4.43	0.039	0.003	0.16	0.688
Small herb (SH)	1	0.004	0.10	0.750	0.023	0.66	0.420	0.001	0.02	0.885	0.001	0.08	0.780
Tall herb (TH)	1	0.219	**5.81**	**0.018**	0.037	1.08	0.303	0.027	0.76	0.385	0.029	1.56	0.216
Residuals	73	0.040			0.035			0.036			0.018		

Table 9. Source of variation in each PCO for bryophyte species presence/absence in 2008. Each PCO is based on Jaccard similarity.

Source of variation		**PCO1**			**PCO2**			**PCO3**			**PCO4**		
	df	MS	F	p	MS	F	p	MS	F	p	MS	F	p
Block	3	0.102	2.50	0.065	0.128	6.41	0.001	0.063	3.16	0.029	0.052	2.81	0.045
Bare ground	1	0.221	**5.45**	**0.022**	0.010	0.51	0.476	0.005	0.27	0.604	0.020	1.06	0.306
SR (log-linear)	1	0.257	**6.33**	**0.014**	0.158	**7.91**	**0.006**	0.220	**10.94**	**0.001**	<0.001	0.01	0.930
FG (linear)	1	0.007	0.16	0.691	0.001	0.04	0.834	0.110	**5.48**	**0.022**	0.022	1.16	0.284
Legume (LE)	1	0.527	**15.31**	**<0.001**	0.212	**12.10**	**0.001**	<0.001	<0.01	0.971	0.046	2.49	0.119
Grass (GR)	1	0.872	**29.10**	**<0.001**	0.053	2.71	0.104	<0.001	<0.01	0.964	0.060	3.32	0.072
Small herb (SH)	1	0.004	0.09	0.769	0.033	1.69	0.198	0.038	1.90	0.172	<0.001	<0.01	0.992
Tall herb (TH)	1	0.060	1.47	0.228	0.003	0.15	0.699	0.035	1.77	0.187	0.001	0.04	0.839
Residuals	75	0.041			0.020			0.020			0.019		

Table 10. Source of variation in each PCO for bryophyte species abundance in 2008. Each PCO is based on Bray-Curtis distances

Source of variation		**PCO1**			**PCO2**			**PCO3**			**PCO4**		
	df	MS	F	p	MS	F	p	MS	F	p	MS	F	p
Block	3	0.156	2.10	0.107	0.074	1.80	0.154	0.011	0.36	0.785	0.020	0.76	0.521
Bare ground	1	0.305	**4.12**	**0.046**	0.033	0.80	0.374	0.003	0.10	0.755	0.087	3.35	0.071
SR (log-linear)	1	1.225	**16.55**	**<0.001**	0.615	**15.03**	**<0.001**	0.005	0.17	0.682	0.002	0.09	0.769
FG (linear)	1	0.001	0.01	0.937	0.085	2.07	0.154	0.017	0.58	0.450	0.009	0.34	0.560
Legume (LE)	1	1.220	**20.54**	**<0.001**	0.490	**13.93**	**<0.001**	0.008	0.26	0.609	0.104	4.14	0.045
Grass (GR)	1	1.228	**20.72**	**<0.001**	0.438	**12.21**	**0.001**	0.145	5.10	0.027	0.113	4.55	0.036
Small herb (SH)	1	0.021	0.28	0.599	0.011	0.26	0.609	0.076	2.59	0.111	0.011	0.43	0.513
Tall herb (TH)	1	0.015	0.20	0.660	0.003	0.06	0.807	0.036	1.21	0.275	0.009	0.34	0.562
Residuals	75	0.074			0.041			0.030			0.026		

Mantel correlations were conducted for seven different relationships between matrices. The correlation for the presence/absence of vascular plants against the presence/absence of bryophytes in 2006 was analysed with the Jaccard similarity metric. The standardized Mantel statistic of the correlation was r = 0.062242, p = 0.013000. Observed Z (0.466876+04) was greater than average Z from randomized runs (0.466269+04) indicating a positive association between vascular plant presence/absence and bryophyte presence/absence. The correlation for the abundance of vascular plants against the presence/absence of mosses in 2008 was analysed with Bray-Curtis distance. The standardized Mantel statistic of the correlation was r = 0.100558, p = 0.001000. Observed Z (0.532347+04) was greater than average Z from randomized runs (0.531251 +04) indicating a positive association between the abundance of vascular plants and bryophyte presence/absence. A mantel correlation

was also run to look at the relationship between the presence/absence of bryophytes in 2006 and 2008, this was analysed using the Jaccard similarity metric, and plots without bryophytes in 2006 were excluded. The standardized Mantel statistic of this correlation was r = 0.204056, p = 0.001000. Observed Z (0.359674+04) was greater than average Z from randomized runs (0.355879+04) indicating a positive association between the presence of bryophytes in 2006 and those in 2008. Two correlations were run that included measures of vascular plant litter in 2008. The correlation for the presence/absence of bryophytes in 2008 (Bray-Curtis distance) vs. total cover litter (squared euclidian) produced the standardized Mantel statistic r = 0.049267, p = 0.192000. Observed Z 0.753908+06) was greater than average Z from randomized runs (0.733852+06) indicating a positive association, although not a significant one, between bryophyte presence and the cover or vascular plant litter. Opposing this, the correlation for the abundance of bryophytes (Bray-Curtis distance) vs. total cover litter (squared euclidian) produced the standardized Mantel statistic r = -0.007942, p = 0.493000. Observed Z (0.833286+06) was less than average Z from randomized runs (0.836205+06) indicating a negative association between bryophyte abundance and the cover of vascular plant litter. The correlation for the presence/absence of bryophytes in 2008 (Bray-Curtis distance) vs. vascular plant cover sown (squared euclidian) produced the standardized Mantel statistic r = 0.123504, p = 0.003000. Observed Z (0.460189+07) was greater than average Z from randomized runs (0.445619+07) indicating a positive association between bryophyte presence and the cover of sown vascular plants. Similarly, the correlation for the abundance of bryophytes in 2008 (Bray-Curtis distance) vs. the total cover of vascular plant species (squared euclidian) produced the standardized Mantel statistic r = 0.158229, p = 0.001000. Observed Z (0.528870+07) was greater than average Z from randomized runs (0.507491+07) indicating a positive association between bryophyte abundance and the cover of vascular plants.

Discussion

Bryophytes and biodiversity

The impact that biodiversity has on ecosystem services has commonly been explored by quantifying the relationship between vascular plant species richness and productivity, particularly so in grassland communities (Hector et al. 1999, Tilman et al. 2001, Balvanera et al. 2006, Cardinale et al. 2006). Biodiversity in these experiments has been shown to have positive effects on productivity (Hector et al. 1999, Tilman et al. 2001, Cardinale et al. 2006) and other ecosystem services associated with nutrient cycling (Balvanera et al. 2006). The diversity of vascular plants has also been shown to positively influence the diversity of other trophic levels (Balvanera et al. 2006) and the interaction networks of multiple trophic levels (Scherber et al. 2010). These results have been used to justify precautionary approaches to biodiversity management. But biodiversity is not limited to vascular plants, even in grassland systems, and other taxonomic groups of plants like bryophytes are seldom considered in these studies (Ingerpuu et al. 2005).

Our results support previous studies that have found a negative correlation between increasing metrics of vascular plant productivity and bryophyte species richness or composition (Virtanen et al. 2000, During and Lloret 2001, Aude and Ejrnæs 2005, Hejcman et al. 2010). Across the gradient of increasing sown vascular plant species richness the species richness of bryophytes decreased threefold in 2006, and by a third in 2008. The majority of the variation in this reduction was explained by vascular plant cover, this indicates it is the increase in primary productivity resulting from higher vascular plant species richness which restricts the number of bryophytes that can establish in a plant community. Vascular plants then delimit bryophyte establishment either by limiting available space or by competing for similar resources. The discussion continues as to whether or not bryophyte species respond to the same abiotic variables that vascular plants do (Pharo et al. 1999, Ejrnæs & Poulsen 2001b). In certain habitats, for example oligotrophic mires and boreal forests, the two taxonomic groups have been shown to respond disparately to environmental gradients (Ejrnæs & Poulsen 2001b). The reason could be that the nature of this relationship may be related to scale. Landscape scale factors like moisture availability might elicit the same

response from vascular plants and bryophytes resulting in their species richness levels being significantly correlated (Pharo et al. 1999). Certainly we have found positive correlations between matrices of the two taxonomic groups. If we focus particularly on acrocarpous bryophyte species, we know that the proportional representation of these in the overall bryophyte sample responded significantly negatively to sown species richness, even when plant cover was included in the model before the experimental factors. Bare ground plots also contained a significantly higher number of acrocarpous bryophytes, these two results taken together suggest it is the availability of more open space, which controls the establishment of a greater number of these species.

Both the presence of legumes in a community and the presence of grasses explained significant amounts of the variation in bryophyte species richness. In 2006 the presence of grass species facilitated bryophyte species richness, and in 2008 this had a negative effect. In both years the presence of legumes negatively influenced bryophyte species richness. Examination of matrices of total bryophyte presence/absence and abundance with PCO analyses further demonstrates the important of grass and legume species in structuring bryophyte communities. There is starkly little information in the literature examining the relationship between bryophytes and grass and legume species. Analysis of single grass and legume species has shown bryophyte species to increase with increasing vascular plant cover, a facilitative effect of improved microclimate, however such experiments with isolated pairs of species are some distance from studies in established plant communities (Ingerpuu et al. 2003). The facilitation of bryophyte species richness by grasses in 2006 is unexpected, pleurocarpous species were expected to be in greater numbers in communities dominated by grasses, as they rely more on dry and wet deposition (Richardson 1981, Bates 2000). Pleurocarpous species are both less diverse and slower to colonise than acrocarpous species therefore species richness should be lower in these communities (Cornish 1954, Gimingham & Birse 1957, Watson 1960, Richardson 1981, Bates 2000). From analyses of individual bryophyte species we know that those which did respond positively to the presence of grass species were pleurocarpous. One possible explanation is that in grass communities bryophytes find a more optimal trade-off between moisture retention and availability of resources like light. Previous work in the

Jena Experiment has demonstrated that legumes have a strong positive impact on aboveground community biomass whereas grasses have on average no significant effect (Marquard et al. 2009). By increasing ambient moisture without reducing other resources grasses may facilitate bryophytes. Many bryophyte species are shade tolerant (Bates 2000) yet grassland bryophytes are known to need relatively high light levels (Rincon & Grime 1989). The decrease in bryophyte species richness in legume communities could be a function of reduced light availability. Alternatively it is a result of nitrogen fertilisation by legumes. Nitrogen fertilisation could affect bryophytes in a number of ways. Bryophyte species richness could be reduced in legume-rich plots as other vascular plants and microbes may express a greater competitive ability. This is unlikely, at least the competitive struggle against other vascular plants, as bryophytes have been shown to have both very high growth rates and plasticity (Furness and Grime 1982, Rincon & Grime 1989). It is plausible that if N inputs from legumes were very high bryophytes could suffer from toxic fertilisation, as a number of bryophyte species are sensitive to N fertilisation (Virtanen et al. 2000, Aude and Ejrnæs 2005, Hejcman et al. 2010). Nitrogen inputs into the soil should be more likely to influence acrocarpous bryophyte species, although there is evidence that pleurocarpous species also uptake nutrients from substrate (Bates 2000). Regardless of bryophyte growth form, both types would be subject to N in solute if it was deposited onto the bryophyte layer as throughfall from the vascular plant canopy. However this is also unlikely as Oelmann et al. (2007) working in the Jena experiment found lower N concentrations in throughfall from the vascular plant canopy of communities containing legumes.

Changes between years may be a result of variation in annual precipitation, or on-going dispersal processes from nearby meadows. Ingerpuu & Kupper (2007) showed fluctuations in bryophyte cover and the cover of dominant bryophyte species were subject to changes in annual precipitation, with cover being highest in years with higher precipitation. But bryophyte colonisation of former arable sites can be very slow. In chalk grasslands in England acrocarpous bryophyte species were observed to colonise abandoned arable land very quickly, while the first pleurocarpous species took 2 years to arrive (Cornish 1954). One final explanation of the difference between the two years relates to the management of the field site, effect sizes are known to be

weaker in biodiversity experiments when manipulations are less well controlled (Balvanera et al. 2006). The resources available to maintain the experimental manipulations were reduced between 2006 and 2008.

Bryophyte richness varied significantly between blocks in this experiment, block was an experimental construct used to randomise the position of vascular plant species richness levels. The significance of block most likely relates to the colonisation processes of bryophytes, which are slow for some species, particularly pleuocarpous species, the proximity of the nearest meadow, and hence spore source has most likely driven this pattern. In both years bryophyte richness was highest in block 1, the most easterly of the blocks, and also that with the closest proximity to the Saale river.

Bryophyte cover reflected similar patterns to species richness in terms of the response to grass and legume presence in a community. Bryophyte cover was significantly higher in communities containing higher relative proportions of sown grass species. Bryophyte cover was on average almost double in communities containing grasses, and more than a third less in communities containing legumes. The mechanism of grasses facilitating bryophyte cover and legumes negating bryophyte cover are likely to be similar to that controlling bryophyte richness. The amount of variation in bryophyte cover explained by grasses is greater than the relative amount of variation in bryophyte species richness, this could indicate increased vegetative growth which lends support to the argument that the abiotic conditions under the canopy of a community with grass species facilitate growth of bryophytes. Although it was not a significant result, the trend between bryophyte cover and sown species richness indicated a vascular plant richness threshold at which point the increasing abiotic benefits to a bryophyte began reversing. Once again the pattern of increased cover without species richness increasing suggests abiotic controls on vegetative growth and other factors delimiting species richness.

Management and conservation

There is strong evidence that in recent times the bryophyte flora of some European grassland has been disrupted and reduced (van Tooren et al. 1990). The cause of this depletion is mostly agricultural, and due to the rationalization and

abandonment of traditionally managed grasslands (van Tooren et al. 1990, Peintinger & Bergamini 2006). The species selected when the Jena experiment was established were chosen so that resulting communities would resemble semi-natural species-rich mesophilic grassland, akin to a Molinio-Arrhenatheretea meadow (Ellenberg 1988). By including plots that were assigned succession and bare ground treatments it is possible to draw comparison to neighboring meadows about the colonization of bryophyte species. Many of the acrocarpous species that we found are typical for disturbed habitats and arable fields, which is to be expected as the reversion of bryophyte composition from arable land to grassland is slow (Cornish 1954, Watson 1960). The management intensity of communities included in the vascular plant species richness gradient decreases as a function of sown species richness. Monocultures and bare ground plots for example are the most disturbed, in terms of weeding and herbicide application, as such they have more open space and resources available for colonization of acrocarpous bryophytes. On the other end of the spectrum, the vascular plant communities sown with 60 species and those that were allowed to undergo succession contain the lowest number of bryophytes, although in the latter succession has included woody species that might further reduce resource availability to bryophytes. In both of these vascular plant community types the composition of bryophyte communities is much different from those of the control meadows indicating either how low the dispersal ability of bryophyte species are from surrounding meadows, or a difference in abiotic variables. This is a salient point for restoration of meadow vegetation. Similarly, bryophyte cover was far higher in control meadows than in all experimental plots, indicating again an abiotic disparity or that a much longer time is required to establish bryophyte biomass.

Summary

Bryophyte species richness responds negatively to increasing vascular plant species richness mostly as a result of the increased cover of the vascular plant community. This demonstrates that not all components of a plant community respond positively to increased species richness; in a grassland system it is therefore possible that advocacy of increasing species richness of one taxonomic group will reduce richness of another.

The presence of grass species in a community facilitates bryophyte species richness and cover and is most likely a result of optimised abiotic conditions for bryophyte establishment and growth. Contrary to this, the presence of legumes in a plot has a negative effect on both bryophyte species richness and cover; this could be an abiotic effect, or the result of toxic fertilisation. The nature of this experiment also permitted insight into dispersal and colonisation processes of bryophytes which could be important for meadow restoration.

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Appendix A: Bryophyte species list.

Amblystegium serpens (Hedw.) Schimp.
Barbula hornschuchiana Schultz
(syn. *Pseudocrossidium hornschuchianum* (Schultz) R.H. Zander)
Barbula unguiculata Hedw.
Brachythecium rutabulum (Hedw.) Schimp.
Bryum argenteum Hedw.
Bryum bicolor complex
Bryum capillare complex
Bryum klinggraeffii Schimp.
Bryum rubens complex
Bryum spp.
Calliergonella cuspidata (Hedw.) Loeske
Ceratodon purpureus (Hedw.) Brid.
Dicranella staphylina H. Whitehouse
Dicranella varia (Hedw.) Schimp.
Didymodon spp./Pseudocrossidium spp.
Ephemerum recurvifolium (Dicks.) Boulay.
Eurhynchium hians (Hedw.) Sande Lac.
(syn. *Oxyrrhynchium hians* (Hedw.) Loeske)
Fissidens taxifolius Hedw.
Funaria hygrometrica Hedw.- *Physcomitrium pyriforme* (Hedw.) Brid.
Funaria spp.
Phascum cuspidatum Schreb. Ex Hedw.
Plagiomnium affine (Blandow ex Funck) T.J.Kop.
Plagiomnium undulatum (Hedw.) T.J.Kop.
Pottia davalliana (Sm.) C.E.O.Jensen.
Pottia intermedia (Turner) Fürnr.
Pottia lanceolata (Hedw.) Müll.Hal.
Pottia spp.
Weissia brachycarpa (Nees & Hornsch.) Jur. - *Weissia controversa* Hedw.
Weissia longifolia Mitt.
Weissia spp.

The three pairs of species in this list result from occasions where determination of bryophytes to species level was not possible because of the absence of sporophyte material from a sample. Through-out the paper only the first species in a pair has been referred to. Additionally two species in the genus *Dicranella* were identified, where it was not possible to differentiate between the two the sample has been designated *Dicranella* spp. For several other samples it was not possible to determine *Pottia*, *Weissia* or *Bryum* to the species-level because the sporophyte was not sufficiently developed, in these situations the epithet spp. has been used.

Appendix B: Supporting tables and figures.

Table B1. ANOVA output of linear model with the CV of bryophyte species richness as the dependent variable and the experimental factors and time as explanatory variables. Explanation of abbreviated names in table: Df (degrees of freedom), SS (sums of squares), MS (mean squares), F (F value), P (P value), SVP (sown vascular plant). Significance codes (S) for P value: 0.001 = ***, 0.01 = **, 0.05 = *.

effect	Df	SS	MS	F	P	S
block	3	1.2062	0.4021	2.4365	0.071355	.
bare ground	1	0.6397	0.6397	3.8767	0.052704	.
SVP cover	*1*	*3.8391*	*3.8391*	*23.2656*	*7.36E-06*	***
SVP species richness	5	1.5483	0.3097	1.8766	0.108659	
SVP functional group richness	4	1.1151	0.2788	1.6894	0.1615	
year	1	0.163	0.163	0.9875	0.323583	
plot	*72*	*21.3359*	*0.2963*	*1.7958*	*0.006584*	**
year x SVP species richness	6	1.089	0.1815	1.0999	0.370603	
year x SVP functional group richness	4	0.7593	0.1898	1.1504	0.339755	
residuals	74	12.2109	0.165			

Table B2. ANOVA output of linear model with bryophyte species richness as the dependent variable and the experimental factors, time, and the sown relative proportion of legume species as explanatory variables. Explanation of abbreviated names in table: Df (degrees of freedom), SS (sums of squares), MS (mean squares), F (F value), P (P value), SVP (sown vascular plant), SRP (sown relative proportion). Significance codes (S) for P value: 0.001 = ***, 0.01 = **, 0.05 = *.

effect	Df	SS	MS	F	P	S
block	*3*	*73.26*	*24.419*	*6.7074*	*0.000458*	***
bare ground	1	7.81	7.806	2.1442	0.147341	
SVP species richness	*5*	*119.89*	*23.979*	*6.5866*	*4.03E-05*	***
SVP functional group richness	4	31.3	7.824	2.1491	0.083144	.
SRP legume species	*1*	*19.8*	*19.801*	*5.439*	*0.022415*	*
year	*1*	*105.96*	*105.959*	*29.1053*	*7.89E-07*	***
plot	*71*	*505.32*	*7.117*	*1.955*	*0.002359*	**
year x SVP species richness	6	16.17	2.696	0.7405	0.618739	
year x SVP functional group richness	4	5.88	1.469	0.4036	0.805477	
year x SRP legume species	1	13.09	13.088	3.5951	0.061853	.
residuals	74	269.4	3.641			

Table B3. ANOVA output of linear model with bryophyte species richness as the dependent variable and vascular plant per cent cover and the experimental factors, time, and the sown relative proportion of legume species as explanatory variables. Explanation of abbreviated names in table: Df (degrees of freedom), SS (sums of squares), MS (mean squares), F (F value), P (P value), SVP (sown vascular plant), SRP (sown relative proportion). Significance codes (S) for P value: 0.001 = ***, 0.01 = **, 0.05 = *.

effect	Df	SS	MS	F	P	S
block	3	73.26	24.419	6.6345	0.000503	***
bare ground	1	7.81	7.806	2.1209	0.149585	
SVP cover	1	171.22	171.22	46.5202	2.24E-09	***
SVP species richness	5	41.7	8.339	2.2657	0.056716	.
SVP functional group richness	4	32.38	8.096	2.1997	0.077366	.
SRP legume species	1	11.38	11.375	3.0907	0.082933	.
year	1	31.83	31.834	8.6493	0.004381	**
plot	71	498.37	7.019	1.9071	0.003352	**
year x SVP species richness	6	12.01	2.002	0.5439	0.773095	
year x SVP functional group richness	4	5.92	1.479	0.4019	0.806673	
year x SRP legume species	1	13.33	13.331	3.6219	0.060965	.
residuals	73	268.68	3.681			

Table B4. ANOVA output of linear model with bryophyte species richness as the dependent variable and the experimental factors, time, and the contrast of communities sown with and without grass species as explanatory variables. Explanation of abbreviated names in table: Df (degrees of freedom), SS (sums of squares), MS (mean squares), F (F value), P (P value), SVP (sown vascular plant), SRP (sown relative proportion). Significance codes (S) for P value: 0.001 = ***, 0.01 = **, 0.05 = *.

effect	Df	SS	MS	F	P	S
block	*3*	*73.26*	*24.419*	*7.1247*	*0.000287*	***
bare ground	1	7.81	7.806	2.2777	0.135508	
SVP species richness	*5*	*119.89*	*23.979*	*6.9965*	*2.11E-05*	***
SVP functional group richness	4	31.3	7.824	2.2828	0.068359	.
grass contrast	*1*	*30.46*	*30.458*	*8.8868*	*0.003886*	**
year	*1*	*105.96*	*105.959*	*30.9163*	*4.06E-07*	***
plot	*71*	*494.67*	*6.967*	*2.0328*	*0.001407*	**
year x SVP species richness	6	16.17	2.696	0.7866	0.583234	
year x SVP functional group richness	4	5.88	1.469	0.4287	0.78745	
year x grass contrast	*1*	*28.87*	*28.869*	*8.4231*	*0.004878*	**
residuals	74	253.62	3.427			

Table B5. ANOVA output of linear model with bryophyte species richness as the dependent variable and vascular plant per cent cover and the experimental factors, time, and the contrast of communities sown with and without grass species as explanatory variables. Explanation of abbreviated names in table: Df (degrees of freedom), SS (sums of squares), MS (mean squares), F (F value), P (P value), SVP (sown vascular plant), SRP (sown relative proportion). Significance codes (S) for P value: 0.001 = ***, 0.01 = **, 0.05 = *.

effect	Df	SS	MS	F	P	S
block	*3*	*73.26*	*24.419*	*7.0333*	*0.000322*	***
bare ground	1	7.81	7.806	2.2485	0.138061	
SVP cover	*1*	*171.22*	*171.22*	*49.3171*	*9.46E-10*	***
SVP species richness	*5*	*41.7*	*8.339*	*2.4019*	*0.045003*	*
SVP functional group richness	4	32.38	8.096	2.3319	0.063744	.
grass contrast	*1*	*30*	*30.002*	*8.6415*	*0.004398*	**
year	*1*	*28.23*	*28.226*	*8.13*	*0.005658*	**
plot	*71*	*483.35*	*6.808*	*1.9609*	*0.002357*	**
year x SVP species richness	6	12.01	2.002	0.5766	0.747734	
year x SVP functional group richness	4	5.92	1.479	0.4261	0.789342	
year x grass contrast	*1*	*28.57*	*28.568*	*8.2286*	*0.005388*	**
residuals	73	253.44	3.472			

Table B6. ANOVA output of linear model with bryophyte species richness as the dependent variable and the experimental factors, time, and the contrast of communities sown with and without legume species as explanatory variables. Explanation of abbreviated names in table: Df (degrees of freedom), SS (sums of squares), MS (mean squares), F (F value), P (P value), SVP (sown vascular plant), SRP (sown relative proportion). Significance codes (S) for P value: 0.001 = ***, 0.01 = **, 0.05 = *.

effect	Df	SS	MS	F	P	S
block	*3*	*73.26*	*24.419*	*6.8431*	*0.000393*	***
bare ground	1	7.81	7.806	2.1876	0.143367	
SVP species richness	*5*	*119.89*	*23.979*	*6.7199*	*3.26E-05*	***
SVP functional group richness	4	31.3	7.824	2.1926	0.078022	.
legume contrast	*1*	*33.08*	*33.076*	*9.2693*	*0.003226*	**
year	*1*	*105.96*	*105.959*	*29.6943*	*6.35E-07*	***
plot	*71*	*492.05*	*6.93*	*1.9422*	*0.002568*	**
year x SVP species richness	6	16.17	2.696	0.7555	0.607115	
year x SVP functional group richness	4	5.88	1.469	0.4118	0.799631	
year x legume contrast	*1*	*18.43*	*18.432*	*5.1654*	*0.025946*	*
residuals	74	264.06	3.568			

Table B7. ANOVA output of linear model with bryophyte species richness as the dependent variable and vascular plant per cent cover and the experimental factors, time, and the contrast of communities sown with and without legume species as explanatory variables. Explanation of abbreviated names in table: Df (degrees of freedom), SS (sums of squares), MS (mean squares), F (F value), P (P value), SVP (sown vascular plant), SRP (sown relative proportion). Significance codes (S) for P value: 0.001 = ***, 0.01 = **, 0.05 = *.

effect	Df	SS	MS	F	P	S
block	*3*	*73.26*	*24.419*	*6.7528*	*0.00044*	***
bare ground	1	7.81	7.806	2.1588	0.146056	
SVP cover	*1*	*171.22*	*171.22*	*47.3496*	*1.73E-09*	***
SVP species richness	5	41.7	8.339	2.3061	0.052962	.
SVP functional group richness	4	32.38	8.096	2.2389	0.073054	.
legume contrast	*1*	*24.31*	*24.311*	*6.7231*	*0.011491*	*
year	*1*	*32.13*	*32.125*	*8.884*	*0.003906*	**
plot	*71*	*485.14*	*6.833*	*1.8896*	*0.003758*	**
year x SVP species richness	6	12.01	2.002	0.5536	0.765616	
year x SVP functional group richness	4	5.92	1.479	0.4091	0.801549	
year x legume contrast	*1*	*18.04*	*18.037*	*4.988*	*0.028588*	*
residuals	73	263.97	3.616			

Table B8. ANOVA output of linear model with the proportion of acrocarpic bryophyte species as the dependent variable and vascular plant per cent cover and the experimental factors, time, and the sown relative proportion of grass species as explanatory variables. Explanation of abbreviated names in table: Df (degrees of freedom), SS (sums of squares), MS (mean squares), F (F value), P (P value), SVP (sown vascular plant), SRP (sown relative proportion). Significance codes (S) for P value: 0.001 = ***, 0.01 = **, 0.05 = *.

effect	Df	SS	MS	F	P	S
block	*3*	*0.6358*	*0.21193*	*3.4815*	*0.020091*	*
bare ground	*1*	*0.3392*	*0.33919*	*5.5722*	*0.020922*	*
SVP cover	*1*	*1.0282*	*1.02822*	*16.8914*	*0.000102*	***
SVP species richness	*5*	*1.5295*	*0.30591*	*5.0254*	*0.000518*	***
SVP functional group richness	4	0.0684	0.01709	0.2808	0.889499	
SRP grass species	*1*	*0.4305*	*0.43047*	*7.0717*	*0.009619*	**
year	1	0.0528	0.05275	0.8666	0.354963	
plot	*71*	*6.7121*	*0.09454*	*1.553*	*0.031687*	*
year x SVP species richness	6	0.533	0.08884	1.4595	0.204194	
year x SVP functional group richness	4	0.1257	0.03142	0.5162	0.72401	
year x SRP grass species	1	0.0678	0.06778	1.1135	0.294794	
residuals	73	4.4437	0.06087			

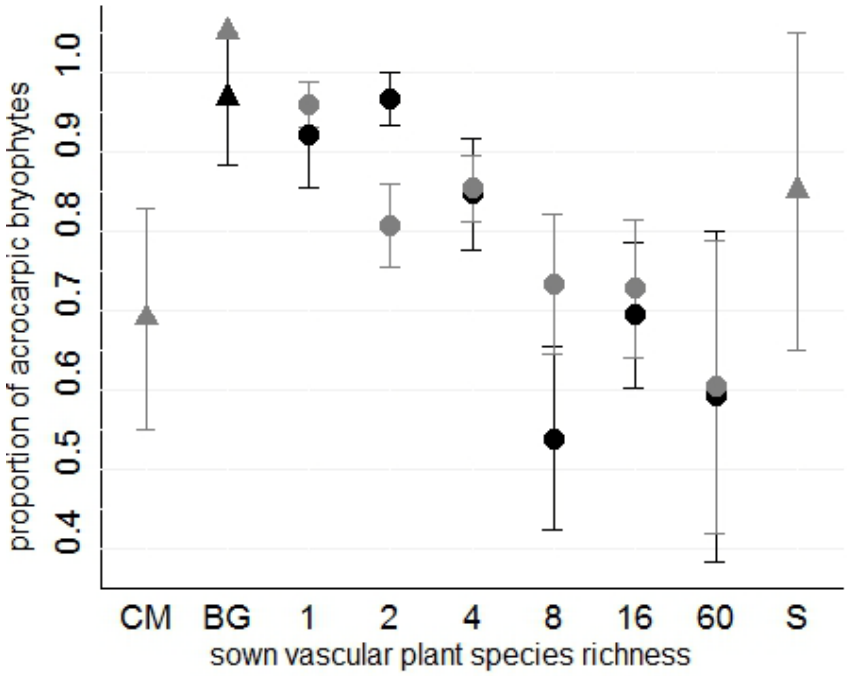

Figure B1. Mean and the standard error of the proportion of acrocarpic bryophyte species per plot in 2006 in black ( ) and 2008 in grey ( ). Circular points ( ) correspond to plots that comprise a level of the vascular plant species richness gradient, triangular points ( ) correspond to plots that do not comprise a level of the vascular plant species richness gradient. Statistical analyses included only those points in the vascular plant species richness gradient in addition to the bareground (BG) plots. CM denotes control meadow plots, S denotes mown succession plots.

Table B9. ANOVA output of linear model with the proportion of acrocarpic bryophyte species as the dependent variable and vascular plant per cent cover and the experimental factors, time, and the contrast of communities sown with and without grass species as explanatory variables. Explanation of abbreviated names in table: Df (degrees of freedom), SS (sums of squares), MS (mean squares), F (F value), P (P value), SVP (sown vascular plant), SRP (sown relative proportion). Significance codes (S) for P value: 0.001 = ***, 0.01 = **, 0.05 = *.

effect	Df	SS	MS	F	P	S
block	3	0.6358	0.21193	3.4796	0.020137	*
bare ground	1	0.3392	0.33919	5.5692	0.020955	*
SVP cover	1	1.0282	1.02822	16.8823	0.000103	***
SVP species richness	5	1.5295	0.30591	5.0226	0.000521	***
SVP functional group richness	4	0.0684	0.01709	0.2807	0.889596	
grass contrast	1	0.5846	0.58464	9.5992	0.002763	**
year	1	0.0464	0.04635	0.7611	0.385858	
plot	71	6.5644	0.09246	1.518	0.039114	*
year x SVP species richness	6	0.533	0.08884	1.4587	0.204474	
year x SVP functional group richness	4	0.1257	0.03142	0.5159	0.724212	
year x grass contrast	1	0.0654	0.06539	1.0736	0.303563	
residuals	73	4.4461	0.06091			

Table B10. ANOVA output of linear model with the mean cover of bryophyte species as the dependent variable and vascular plant per cent cover and the experimental factors, and the sown relative proportion of grass species as explanatory variables. Explanation of abbreviated names in table: Df (degrees of freedom), SS (sums of squares), MS (mean squares), F (F value), P (P value), SVP (sown vascular plant), SRP (sown relative proportion). Significance codes (S) for P value: 0.001 = ***, 0.01 = **, 0.05 = *.

effect	Df	SS	MS	F	P	S
block	3	1889.90	629.98	2.1376	0.1040	
bare ground	1	3.30	3.31	0.0112	0.9159	
SVP cover	1	2.70	2.66	0.0090	0.9245	
SVP species richness	5	1615.70	323.14	1.0964	0.3710	
SVP functional group richness	4	1040.80	260.20	0.8829	0.4792	
SRP grass species	*6*	*5000.10*	*833.35*	*2.8276*	*0.0166*	*
residuals	65	19156.60	294.72			

Chapter 4

Janzen-Connell effects are widespread and strong enough to maintain diversity in grasslands.

Petermann, J.S., Fergus, A.J.F., Turnbull, L.A. & Schmid, B. 2008. *Ecology* **89**: 2399-2406.

Abstract

Crop rotation schemes are believed to work by preventing specialist soil-borne pests from depressing the future yields of similar crops. In ecology, such negative plant–soil feedbacks may be viewed as a type of Janzen-Connell effect, which promotes species coexistence and diversity by preventing the same species from repeatedly occupying a particular site. In a controlled greenhouse experiment with 24 plant species and using soils from established field monocultures, we reveal community-wide soil-based Janzen-Connell effects between the three major functional groups of plants in temperate European grasslands. The effects are much stronger and more prevalent if plants are grown in interspecific competition. Using several soil treatments (gamma irradiation, activated carbon, fungicide, fertilizer) we show that the mechanism of the negative feedback is the buildup of soil pathogens which reduce the competitive ability of nearly all species when grown on soils they have formerly occupied. We further show that the magnitude of the change in competitive outcome is sufficient to stabilize observed fitness differences between functional groups in reasonably large communities. The generality and strength of this negative feedback suggests that Janzen-Connell effects have been underestimated as drivers of plant diversity in temperate ecosystems.

Introduction

A revolution in agriculture occurred when crop rotation was introduced to combat what became known as “soil sickness,” or the faltering productivity of crops sown recurrently on the same site. For example, typical European crop rotations in the nineteenth century involved wheat, barley, turnips, and clover or peas (Overton 1996). These crops belong to what we now recognize as three different functional groups: grasses, forbs, and legumes. These plant functional groups have a taxonomic basis, and as closely related species are likely to share pests and pathogens (Gilbert and Webb 2007), the success of crop rotation schemes could be due to the avoidance of negative soil feedbacks (Bever 1994). Here, we explore negative soil feedbacks among the same three functional groups in natural grassland. If pathogens accumulate in the soil, they

may reduce the chance that a related species will capture the site once a plant dies, potentially leading to natural rotations analogous to those imposed by farmers.

Studies in temperate grasslands have already shown that species can negatively affect the growth of conspecifics via the soil compartment (van der Putten et al. 1993, Bever 1994, De Deyn et al. 2003) and demonstrated a relationship between the size of such negative feedbacks and species abundances (Klironomos 2002). However, much of the work on negative soil feedbacks has focused on exotic invasions and community succession (but see Bever 1994, Olff et al. 2000, Bonanomi et al. 2005). For example, such studies have demonstrated that native–invasive interactions are strongly influenced by soil-mediated feedbacks acting via fungi or other soil organisms (Reinhart and Callaway 2006); although allelochemicals can sometimes be involved (Callaway and Aschehoug 2000). A further substantial part of the plant–soil feedback literature deals with successional dynamics, where negative soil feedbacks help to explain directed species change (van der Putten et al. 1993, van der Putten and Peters 1997, De Deyn et al. 2003).

We studied a native nonsuccessional grassland and examined how negative soil feedbacks can potentially facilitate the coexistence of species and the maintenance of diversity by acting in a similar way to the Janzen-Connell effect (Bever 2003). Janzen (1970) and Connell (1971) suggested that adults, by harboring host-specific predators and herbivores, could locally reduce the recruitment success of conspecific juveniles. However, the importance of the Janzen-Connell effect as a coexistence mechanism remains in question, because it has only been shown to operate for a single or few species within any particular community (Augspurger and Kelly 1984, Condit et al. 1992, Packer and Clay 2000, Bell et al. 2006). The prevalence and strength of the effect was therefore deemed insufficient as a mechanism of diversity maintenance (Gilbert 2005). The Janzen-Connell effect has also been exclusively associated with tropical ecosystems (Freckleton and Lewis 2006) or, very rarely, with temperate forests (Packer and Clay 2000, Hille Ris Lambers et al. 2002). However, negative soil feedbacks involving species-specific pathogens in grasslands can maintain diversity in a fundamentally similar way to Janzen-Connell effects: negative soil feedbacks reduce the chance of conspecific juveniles capturing sites following the death of adults, while

Janzen-Connell effects reduce the chance of conspecific juveniles capturing sites close to existing adults.

We performed a controlled greenhouse experiment using common species from temperate grassland and soil collected from established field plots. We grew each species alone; but in contrast to other studies, we additionally grew each species in competition with other functional groups (Bever 2003). The influence of negative feedbacks on competitive ability has rarely been studied, potentially leading to an underestimation of their magnitude and relevance to natural communities. We concentrated on functional groups because pathogen-related effects may be more likely to maintain diversity at higher phylogenetic levels than at the species level (Gilbert and Webb 2007). In order to explore possible mechanisms behind observed effects, we applied a number of soil treatments that selectively excluded certain groups of potential feedback agents. Finally, we put our measured effect sizes into a community context by modeling how such effects interact with the inevitable fitness differences which exist between species, both within this particular community and more generally.

Materials and methods

Soil origin and preparation

Field monocultures of 24 common European grassland species, eight grasses, eight forbs, and eight legumes (see legend of Fig. 2 and Appendix A: Table A2), were grown for three growing seasons near Zurich, Switzerland (Wacker et al. 2008). In autumn 2005, we removed four subsamples of soil per monoculture, pooled them and added 20% of washed and autoclaved sand.

We subdivided soils into a control and four treatments to investigate the general causes of potential plant–soil feedbacks. The treatments were (1) sterilization by gamma irradiation to remove all soil organisms, (2) fungicide to remove only fungi, (3) activated carbon to remove allelochemicals, and (4) fertilizer to serve as an additional control for nutrient flushes that may result from the killing of soil organisms (Troelstra et al. 2001). Fertilizer-treatment pots received a liquid NPK fertilizer once at the beginning of the experiment. There was no fertilization in any of the other treatments (see Appendix A for more details).

Experiment

The same 24 species that had conditioned the soil in the field were then grouped into eight sets, each containing one forb, one grass, and one legume species (Appendix A: Table A2). Species were reciprocally grown on their own soils ("home") and on soils from the two other species in the set ("away") in the glasshouse. We sowed seeds in monocultures on the respective home and away soils that had been subjected to the five soil treatments. There were five replicates of each combination (1800 0.2-L pots), and we recorded germination percentages after 12–20 days, depending on germination behavior of the species.

For the main experiment, seedlings were transplanted into 0.6-L pots filled with the treated soils. Communities with one of two types of competition were assembled. The first type of competition involved planting three individuals of the same species together on both the home soil and the two away soils within the set, without competition from other species (24 species × 3 soils × 5 soil treatments = 360 pots; Appendix C: Fig. C1). The second competition type involved planting one individual from each of the three species in the set together in the same pot on each of the three soils. Thus, on each soil one species was always growing "at home" while the other two were growing "away" (Appendix A: Table A2). There were three replicates per multi-species combination (8 multispecies sets × 3 soils per set × 5 soil treatments × 3 replicates = 360 pots). When the first plants started to flower after eight weeks, the experiment was stopped, all aboveground plant parts were harvested and weighed after drying at 70°C for 48 hours (see Appendix A for more details).

Data analysis

Dry mass of single plants growing on home soil was divided by the dry mass of single plants growing on away soils to get a proportional measure of feedback that is independent of plant size (in contrast to the measure used by Klironomos (2002)). For example, for species *i*,

$$\text{Feedback}_i = \log\left[\frac{\text{biomass}_i(\text{home})}{\text{biomass}_i(\text{away})}\right]$$

where biomass*i* (home) = biomass of species *i* on its own soil and biomass*i* (away) = biomass of species *i* on soil of species *j* (in the interspecific competition treatment, the average mass of individuals across the three replicates was used). Because each species was grown on two different away soils, each belonging to a different functional group, this resulted in two values of the feedback measure for each species per competition type and soil treatment (480 degrees of freedom in the main experiment). The ratio was log-transformed to achieve normality and homogeneity of variances. At the same time, the log transformation returns zero when there is no difference between home and away soils, and negative values for "negative feedbacks" (biomass at home smaller than biomass away) and vice versa. The log-ratio was then used as the response variable in a mixed-model ANOVA (Table A1). A similar analysis was done with germination percentage instead of biomass for the germination experiment (Table A3).

Modeling

Our intention was to see whether a typical grass, forb, and legume could coexist given the strengths of negative soil feedbacks measured in the experiment. Thus, we modeled a simplified community consisting of three different functional groups, each containing one average or typical species. Rather than use a deterministic framework (Bever 2003), we chose a stochastic formulation to assess the impact of demographic stochasticity on persistence times. We assumed that adult individuals die at rate d and a new individual of species *i* is recruited to fill a site formerly occupied by an individual of type *j* with probability. $Pij = Nicij/\Sigma Nicij$. Here *Ni* is the population size of the *i*th species and *cij* is the competitive weighting for species *i* at a site formerly occupied by species *j*. If $cii < cij$ then negative feedbacks operate, because species are less competitive when recapturing sites which they have formerly occupied.

Suitable values for the competition coefficients can be determined from our experiment, as we can assess the competitive ability of each species against identical competitors on different soil types. We can average over the eight sets to obtain robust "typical" values for each functional group. Suitable values for the death rates, *di* were

estimated from field monocultures of 52 species (the entire original species pool from which our 24 species were randomly selected for this experiment). These values should typify the kind of fitness differences found between the functional groups, although some important processes are inevitably missing.

In addition to simulations including specific values estimated from the data, we also explored a range of other scenarios to examine the general relationships between fitness differences, negative feedback strengths and community size. We used four community sizes (99, 501, 999, and 5001 plant individuals) where each of the three functional groups had equal population sizes in generation 1. A proportion *di* of individuals belonging to species *i* was randomly selected and removed each generation during a single mortality episode (to mimic episodic mortality such as that induced by summer drought or winter cold) followed by a single episode of recruitment restoring the initial community size. We considered the persistence of the three functional groups over the long term (10 000 generations) in two ecological scenarios: (1) all functional groups had the same adult mortality rate $di = 0.2$ for all *i* and (2) there were differences in mortality rates for each functional group of either 10% ($d1 = 0.2$, $d2 = 0.9 \times d1 = 0.18$, and $d3 = 0.9 \times d2 = 0.162$) or 20% ($d1 = 0.2$, $d2 = 0.8 \times d1 = 0.16$, $d3 = 0.8 \times d2 = 0.128$). In each case, the probability of persistence of all three functional groups was calculated from 1000 runs each of 10 000 generations. Dispersal was global so that each functional group had the same chance of arriving at a site, although we analyzed a subset of models with local dispersal (see Appendix A).

Results

We found strong negative plant–soil feedbacks throughout our study community ($F_{1,23} = 35.69$, $P < 0.001$; Appendix A: Table A1). On average, plants produced 30% less biomass when growing on home rather than on away soils (Fig. 1, “Control”), with the effect being considerably more severe when plants were grown in competition with the other two functional groups ($F_{1,23} = 16.68$, $P < 0.001$). In the pots with interspecific competition, 23 out of 24 species suffered a negative feedback (Fig. 2a, left) and plant mass was on average halved on home compared with away soils. In monocultures, fewer species experienced negative feedbacks and the effects were much weaker than

in interspecific competition (Fig. 2a left vs. right). Species from each functional group grew equally well on soil from either of the other two functional groups ($F_{2,22} = 0.47$, P = 0.634). Furthermore, species from each functional group suffered the same magnitude of negative feedback ($F_{2,21} = 0.53$, P = 0.595). Within functional groups, the size of the effect varied between species ($F_{21,215} = 12.86$, P < 0.001). For example, the biomass reduction when growing on home vs. away soils in competition with the other two functional groups ranged from 90% in *Echinochloa crus-galli* to around 4% in *Centaurea jacea*. Only one out of 24 species, *Trifolium incarnatum*, had a higher biomass (+6%) on home soil. When grown in monoculture, the effect size ranged from a 55% biomass reduction (*Hordeum murinum*) to a 25% increase (*Melilotus albus*) on home soil. In contrast to the effects on growth, there was no general home vs. away effect on seedling emergence ($F_{1,23} = 0.24$, P = 0.627; Appendix A: Table A3; Appendix B: Fig. B1).

Our experiment also included soil manipulation treatments designed to investigate potential mechanisms. These treatments differed significantly in their impact ($F_{4,92} = 4.68$, P = 0.002). Gamma irradiation removed the negative feedback almost completely (Fig. 1), particularly when species were grown in competition with the two other functional groups (Fig. 2b left). The fungicide treatment of the soil resulted in a net increase of the negative feedback compared with controls (Fig. 1, Fig. 2c left and right). The activated carbon treatment had little effect in our experiment (Fig. 1, Fig. 2d left and right), and the fertilization treatment reduced the negative feedback effect, although not as effectively as the gamma irradiation (Fig. 1, Fig. 2e left and right).

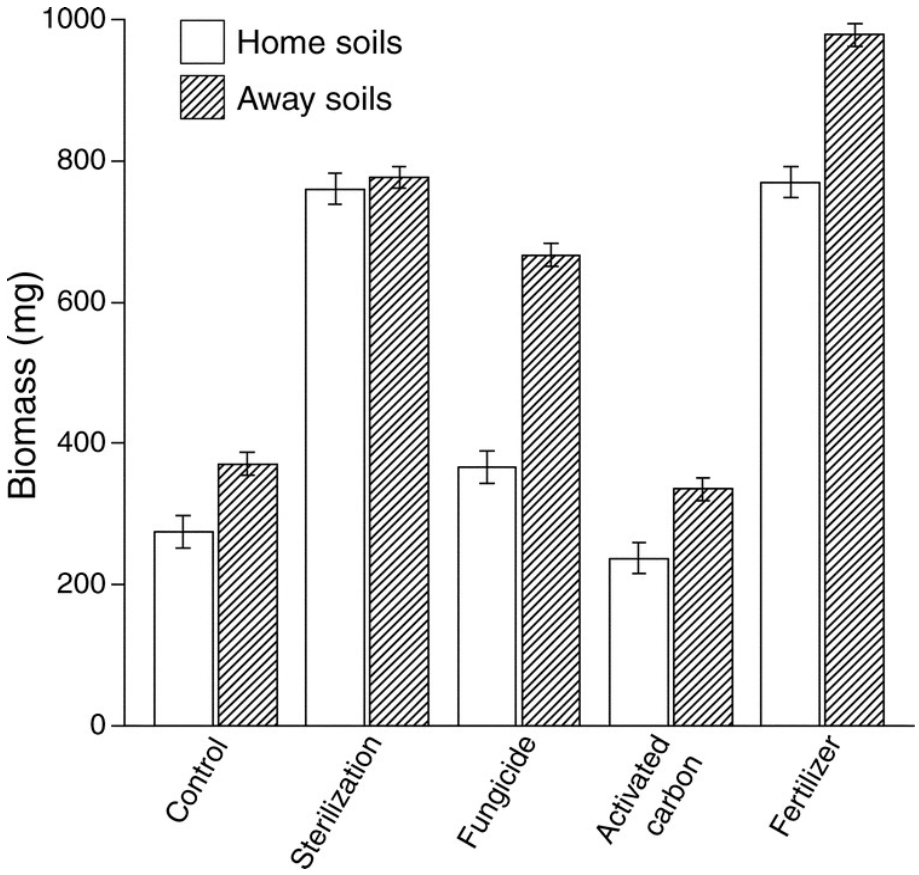

Fig. 1. Absolute biomass per plant individual (mean ± SE) on home soils (open bars) and away soils (hatched bars) for controls and the four soil treatments; data are from monocultures and three-species competition treatments combined. Only soil sterilization eliminates the disadvantage of growing on home soils.

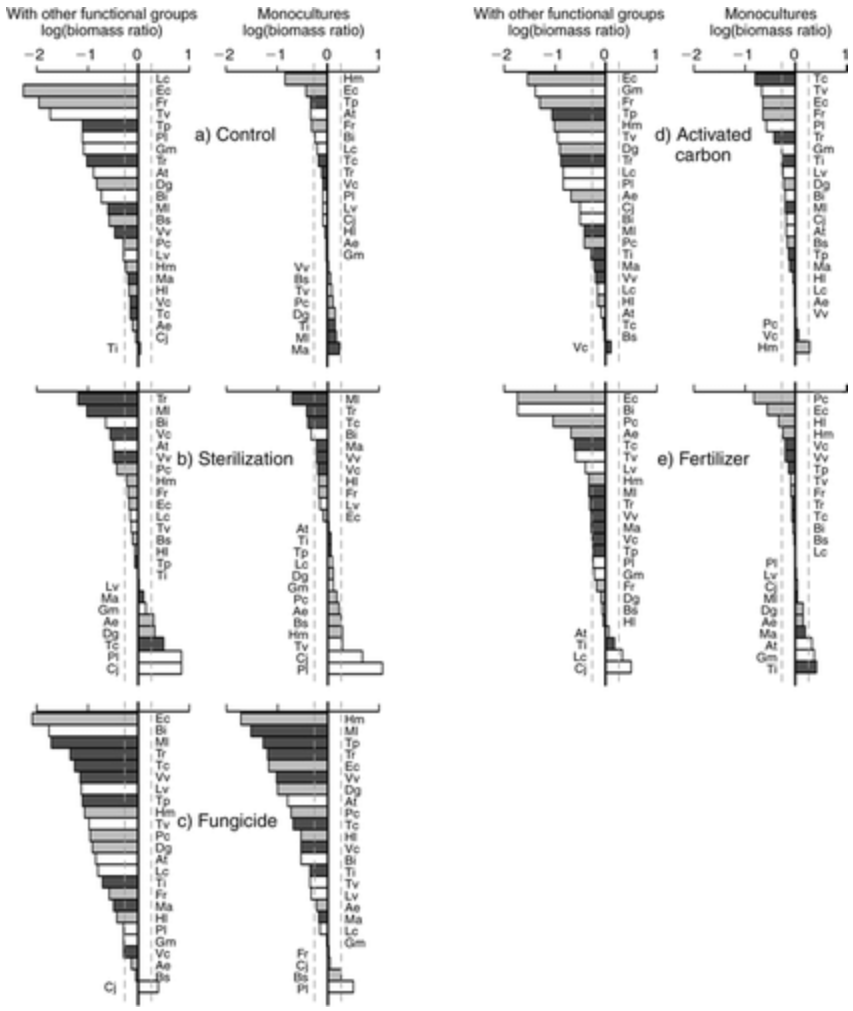

Fig. 2. Mean soil feedbacks for all 24 species of European grassland plants: biomass of individual plants on home soils was divided by biomass of individuals on away soils for each species, then log-transformed. Negative values correspond to a net disadvantage on home soils (negative feedback); positive values to a benefit on home soils (positive feedback). The left-hand column shows plants grown with competition from other functional groups; the right-hand column shows monocultures. Dashed lines show ±SE around zero. Forbs are represented by white bars, grasses by light gray bars, and legumes by dark gray bars. Abbreviations: At, *Arctium tomentosum*; Ae, *Arrhenaterum elatius*; Bi, *Berteroa incana*; Bs, *Bromus sterilis*; Cj, *Centaurea jacea*; Dg, *Dactylis glomerata*; Ec, *Echinochloa crus-galli*; Fr, *Festuca rubra*; Gm, *Galium mollugo*; Hl, *Holcus lanatus*; Hm, *Hordeum murinum*; Lc, *Lepidium campestre*; Lv, *Leucanthemum vulgare*; Ml, *Medicago lupulina*; Ma, *Melilotus albus*; Pc, *Panicum capillare*; Pl, *Plantago lanceolata*; Tv, *Tanacetum vulgare*; Tc, *Trifolium campestre*; Ti, *T. incarnatum*; Tp, *T. pratense*; Tr, *T. repens*; Vc, *Vicia cracca*; Vv, *V. villosa* (Lauber and Wagner 1996). Lc indicates the control (outlier excluded).

Modeling

The model parameters are particularly simple: we can use the same value of c_{ii} for each functional group as the magnitude of the negative feedbacks suffered by each functional group was the same ($F_{2,21} = 0.53$, $P = 0.595$). In addition, we can use a single value of c_{ij} for all i and j, as each functional group grew equally well when "away" on soils belonging to either of the other two functional groups ($F_{2,22} = 0.47$, $P = 0.634$). By setting the competitive weighting when capturing away sites (c_{ij}) to unity for all i and j we can vary the size of the negative feedback on home soils by choosing values for the competitive weighting when trying to capturing home sites (c_{ii}) in the range 0–1. In our experiment, species from all functional groups had roughly half the biomass when grown with the same competitors on home rather than on away soils; therefore we would estimate $c_{ii} = 0.5$ for all i. In the special case of $c_{ii} = 1$, the model becomes neutral and the only force in the community is drift. In contrast, when $c_{ii} = 0$, a species has no chance of recruiting on a home site, and the model is deterministic in the case of two species. However, there will always be stochasticity in the three-species case because species from the remaining two functional groups have an equal chance of capturing any site vacated by the third.

The model revealed that even weak negative soil feedbacks ($c_{ii} \leq 0.9$) lead to stable coexistence when different functional groups have equal fitness ($d_i = d_j$), but that stronger feedbacks are necessary to ensure coexistence when species differ in fitness ($d_i \neq d_j$, Fig. 3a). Fitness differences between species lead to unequal equilibrium population sizes and therefore increase the probability that the functional group with the lowest fitness becomes extinct. Much stronger negative feedbacks are therefore required to reduce fluctuations around the equilibrium and hence stabilize the interaction (Fig. 3a–d). The strength of negative feedback estimated here ($c_{ii} = 0.5$) would stabilize fitness differences among functional groups of around 10%, but not of 20%, even in a large community (5000 individuals; Fig. 3a). Using observed death rates of the three functional groups from field monocultures (legume $d = 0.466$, grass $d = 0.450$, forb $d = 0.364$) reveals that this value ($c_{ii} = 0.5$) is sufficient to ensure persistence of all three functional groups in communities of ≥500 individuals (Fig. 3, dashed lines).

However, this only holds if a sufficiently high proportion of the seeds produced (more than about 50%) disperse away from the parent site (see Appendix D: Fig. D1).

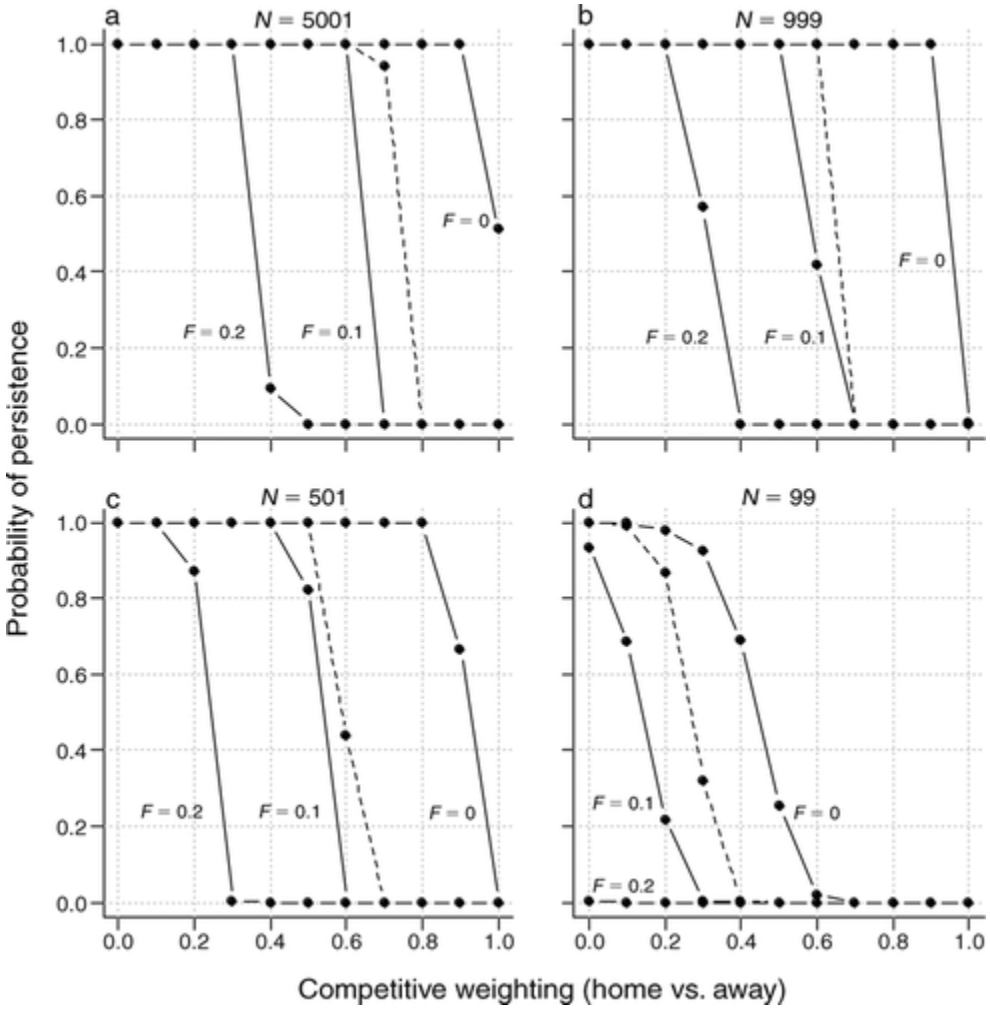

Fig. 3. Community dynamics with and without negative soil feedbacks: in a community of fixed total size (*N*), the probability of all three functional groups persisting for 10000 years decreases as the competitive weighting (home vs. away) increases, and as fitness differences (*F*) between functional groups increase from zero (*F* = 0) to 10% (*F* = 0.1) and 20% (*F* = 0.2). As the community size decreases from ~5000 to ~100 (a–d) a lower weighting (home vs. away) is needed to ensure persistence. Fitness differences are incorporated as differences in death rates. Model simulations using observed fitness differences between functional groups (average death rates over the summer) are also shown (dashed line). The competitive weighting (home vs. away) estimated in this experiment is 0.5.

Discussion

In our communities, feedback effects were strong and pervasive, and species from all three functional groups were similarly disadvantaged when competing for sites which they had formerly occupied. The effects were considerably weaker when plants were grown only with conspecifics, indicating that it is competitive ability that is primarily affected. Negative feedbacks affecting competitive ability, rather than growth in the absence of competition have rarely been directly investigated and this might have lead to a significant underestimation of the incidence and strength of Janzen-Connell effects in natural communities. We also failed to find effects on germination and seedling survival, although many studies of Janzen-Connell effects only examine these measures (Hyatt et al. 2003).

The almost complete removal of the negative feedback across the community by soil sterilization strongly suggests that soil biota were the primary agents causing the observed effects. These soil organisms must be host-specific (Freckleton and Lewis 2006) as generalist pathogens would be expected to affect plants growing at home and away equally. Pathogenic fungi are most often specifically examined in feedback studies and in some cases their effect has been directly demonstrated (Mills and Bever 1998, Packer and Clay 2000, Klironomos 2002). However, pathogenic fungi can be very variable in their host range (Augspurger and Wilkinson 2007) and very often information about host-specificity is lacking (Freckleton and Lewis 2006). In our study, we were unable to attribute the effect to soil fungi. While absolute plant biomass increased on both home and away soils with the addition of fungicide, it increased more strongly on away soils, intensifying the net negative feedback (Fig. 1). The most likely explanation is that generalist pathogenic fungi constrained other, even more detrimental, soil organisms (e.g., bacteria, or specific fungicide-tolerant fungi or fungus-like organisms such as Oomycetes). In a separate experiment, Zeller et al. (2007) showed that the biomass of the species with the greatest negative soil feedback, Echinochloa crus-galli, is reduced by 90% when infected with a cyanide-producing Pseudomonas bacterial strain. Other soil organisms that could be responsible for the negative feedback include nematodes and larger invertebrates such as insect larvae (De Deyn et al. 2003).

In contrast to previous studies which have demonstrated dramatic negative effects caused by allelochemicals released by exotic plant invaders on native plant species (Callaway and Aschehoug 2000), we found no consistent chemically mediated effects. Thus, chemical weapons do not seem to play an important role in structuring communities of native species with a common evolutionary history. The fact that the negative feedback was to some extent reduced in samples treated with fertilizer implies that part of the negative soil feedback could have been due to specific nutrient depletion. This represents abiotic density dependence (Ehrenfeld et al. 2005) and corresponds to predictions from classical resource niche theory (Tilman 1982). On the other hand, fertilizer addition could simply have mitigated the detrimental effect of pathogenic soil organisms by removing nutrient limitation (van der Putten and Peters 1997).

Several studies have examined feedback effects and their impact on community processes using deterministic models and have identified conditions for successful exotic invasion and the coexistence of species (e.g., Eppstein and Molofsky 2007). Here, we investigated the potential consequences of measured feedbacks on the persistence of three species each belonging to a different functional group in communities of different total size. In a community without negative feedbacks, the population dynamics are characterized by pure ecological drift and there is no stable equilibrium (Chesson 2000, Hubbell 2001). Although populations can persist for long periods under drift, this is only true when fitness differences are minimal (Zhang and Lin 1997). When fitness differences are present, stabilizing mechanisms, for example resource niches, are required (Chesson 2000); and the stronger the fitness inequalities, the stronger the stabilizing forces needed (Adler et al. 2007, Harpole and Suding 2007). We show that soil-mediated negative feedbacks can be potent stabilizing forces. The size of the measured feedbacks, coupled with information on typical fitness differences between the functional groups, indicates that negative soil feedbacks could play an important role in the maintenance of functional diversity in grasslands, providing that seeds are dispersed sufficiently far from the parent sites.

In traditional niche theory, the number of species able to coexist in a community increases with the number of niche dimensions (Hutchinson 1978). Recently, this concept of “high-dimensional” coexistence has again gained favor (Clark et al. 2007). Here we show that Janzen-Connell effects could be an important source of niche-dimensionality, with “pathogen niches,” or rather pathogen-free space, providing the resource axes. Similarly, studies of biodiversity and ecosystem functioning often conclude that resource partitioning causes diverse communities to outperform monocultures; however, Janzen-Connell effects could be an equally likely explanation of why monocultures “under perform” compared to mixtures (Mwangi et al. 2007). Soil-mediated Janzen-Connell effects might furthermore be the reason that monocultures are much more easily invaded than mixtures, as has been shown in numerous previous experiments (Hector et al. 2001, Mwangi et al. 2007).

Our results demonstrate that Janzen-Connell effects are widespread among the three major functional groups in European grasslands. Each functional group is consistently disadvantaged when competing for sites that it has formerly occupied, leading to natural rotations of site occupancies, similar to those traditionally imposed by farmers. Under a neutral model, a monoculture functions just as well as a diverse community; but if low-diversity communities quickly accumulate specialist soil pathogens, these depauperate communities may develop the same “soil sickness” which continues to plague some farmers today.

Acknowledgements

We thank G. de Deyn, A. Hector, D.W. Purves, W. van der Putten, and D.A. Wardle for discussions and comments. This research was supported by grant 31-65224.01 from the Swiss National Science Foundation to B. Schmid and L.A. Turnbull. J.S. Petermann and A.J.F. Fergus contributed equally to this work.

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Appendix A. Supplementary materials, methods, and tables.

Soil origin and preparation

We used soils from 24 field monocultures (one per species), each measuring 1.5 × 2 m. We removed four subsamples of soil from the top 25 cm of each of the monocultures, insulated them against peak frosts and stored them outside for three months to mimic seasonal temperature changes. We mixed the soils by sieving (1 cm mesh width), removed stones, cut roots into 1.5 cm pieces and returned them to the soil.

Soils were either (1) sterilized by gamma irradiation (>25 kGray) to remove all soil organisms, (2) received twice the recommended dose of a broad-spectrum fungicide to remove all fungi (Carbendazim; Methyl-benzimidazol-2-ylcarbamat, Sintagro AG, Härkingen, Switzerland, 1.8 g/pot), (3) were mixed with activated carbon (washed with hydrochloric acid, Sigma-Aldrich, Switzerland, 2 % by volume) to remove allelochemicals or (4) were fertilized with a liquid NPK-fertilizer (Gesal, Compo Jardin AG, Allschwil, Switzerland, 110.7 mg/pot N (102.6 mg as carbamide and 8.1 mg as ammonium), 63.6 mg/pot P (as phosphoric acid), 180.0 mg/pot K (as potassium hydroxide) once at the beginning of the experiment. Nutrient concentrations were still significantly higher in the fertilizer treatment compared with the sterilization treatment at the end of the experiment (based on a subset of 9 soil types from 54 mixture pots (replicate pots were pooled): $F_{1,8} = 11.9$, $P < 0.01$ for nitrogen and $F_{1,8} = 63.5$, $P < 0.001$ for phosphorus).

Experiment

The 24 species were grouped into eight sets, each containing one forb, one grass and one legume species (Table A2). Initially, species were grouped into four early- and four mid-successional sets with random assignment of species within functional group and successional stage. The factor "successional stage" was not significant ($F_{1,6} = 0.64$, $P = 0.451$, tested against "set" within the species term) and was dropped from the analysis. We surface-sterilized seeds with 7 % sodium-hypochlorite before the experiment. Plants were grown in the glasshouse under a 15/9h light/dark cycle (minimum light level 400 $\mu Em^{-2}s^{-1}$ during the day) and a mean temperature of 20°C (minimum 15°C, maximum 28°C). We watered all pots manually three times a week to keep soil moisture constant, avoiding any exchange of water between the pots. Pots were randomized every two weeks to remove spatial variation.

Supplementary Modeling: Incorporating local dispersal into the model framework

In the models presented in the main paper, we assume infinite fecundity and global dispersal. To incorporate local dispersal, we first need to make fecundity finite. The model also needs to be spatially explicit, with a grid of N patches which are fully occupied by the three functional groups. Some fraction (F) of the seeds produced by each individual remains within the local patch, while the remainder (1-F) is dispersed to form a global seed rain. All plants reproduce before mortality acts, thus the mean

number of seeds of species *i* arriving in patch *q* at time *t* ($n_{i,q,t}$) follows a Poisson distribution, with mean equal to the sum of the within-patch and global dispersal terms:

$$\overline{n}_{i,q,t} = F.(R_i) + (1-F)R_i.N_i.(1/N)$$

where R_i is the reproductive output of an individual of species *i*. We chose R_i = 100 as this is typical of values found for grassland plants, and creates a suitable degree of stochasticity in the seed inputs. Otherwise the model is the same as described in the main paper with parameter values taken from the experimental and field data, i.e., c_{ii}= 0.5 for all *i*, c_{ij}= 1 for all *i* and *j* and d_{legume} = 0.466, d_{grass}= 0.450, d_{forb}= 0.364. We varied *F* in the range 0 – 1 in steps of 0.1 (*F* = 1 corresponds to full global dispersal). For each value in this range, the mean persistence time of all three functional groups was calculated from 100 runs each of 10,000 generations.

Local dispersal does indeed have dramatic consequences for the persistence of the three functional groups (Fig. D1). Only when $F \leq 0.7$ do the three functional groups persist for 10,000 generations. When *F* = 0.6, the three groups persist on average for around 6,000 years, but with $F \leq 0.5$, the functional group with the lowest fitness only persists on average for 2,000 years (and never persists for 10,000 years). This occurs because seeds are increasingly concentrated in patches where recruitment probability is low. This highlights the importance of dispersal away from the parent site when Janzen Connell effects or negative soil feedbacks operate. This is particularly true when species have unequal fitness, as the species with the lowest fitness must ensure that it disperses seeds into sites where it has a better chance of recruiting.

Supplementary Tables

Table A1. Results from the mixed-model ANOVA for log-ratio of biomass (biomass of individual plants on home soils divided by biomass of individuals on away soils for each species, log-transformed). The species term (bold) is split into one contrast (normal print), the row numbers of the respective error terms are given in the last column (fixed effects are tested against random effects, random effects against the residual).

Source of variation	df	*F*	*P*	Error term
1 Mean	**1**	**35.69**	**<0.001**	**4**
2 Competition	**1**	**16.68**	**<0.001**	**6**
3 Functional group of soil	**2**	**0.47**	**0.634**	**7**
4 Species	**23**	**12.33**	**<0.001**	**11**
4a Functional group	2	0.53	0.595	4b
4b Species	21	12.86	<0.001	11
5 Treatment	**4**	**4.68**	**0.002**	**8**
6 Competition × Species	**23**	**6.55**	**<0.001**	**11**
7 Functional group of soil × Species	**22**	**4.08**	**<0.001**	**11**
8 Species × Treatment	**92**	**10.24**	**<0.001**	**11**
9 Competition × Treatment	**4**	**9.59**	**0.010**	**10**
10 Competition × Species × Treatment	**91**	**1.67**	**0.001**	**11**
11 Residuals	**215**			
Total	478			

Table A2. The 24 species were grouped into eight experimental sets, each containing one grass, one forb and one legume of the same successional stage. Assignment of species to sets within successional stage and functional groups was random. Each species was then grown on home (soil from same species) and away soils (soils from the other two species in the set) either in monoculture or in competition with the other species in its set.

Set	Species	Functional group	Successional stage
1	*Panicum capillare*	GRASS	early
	Lepidium campestre	FORB	
	Trifolium incarnatum	LEGUME	
2	*Bromus sterilis*	GRASS	early
	Arctium tomentosum	FORB	
	Trifolium campestre	LEGUME	
3	*Echinochloa crus-galli*	GRASS	early
	Berteroa incana	FORB	
	Melilotus albus	LEGUME	
4	*Hordeum murinum*	GRASS	early
	Tanacetum vulgare	FORB	
	Vicia villosa	LEGUME	
5	*Arrhenaterum elatius*	GRASS	mid
	Plantago lanceolata	FORB	
	Medicago lupulina	LEGUME	
6	*Holcus lanatus*	GRASS	mid
	Centaurea jacea	FORB	
	Trifolium pratense	LEGUME	
7	*Festuca rubra*	GRASS	mid
	Leucanthemum vulgare	FORB	
	Vicia cracca	LEGUME	
8	*Dactylis glomerata*	GRASS	mid
	Galium mollugo	FORB	
	Trifolium repens	LEGUME	

Table A3. Results from a mixed-model ANOVA of log-ratio of seedling emergence (seedling emergence probability on home soils divided by seedling emergence probability on away soils, ratio log-transformed), random effects were the species term and its interaction, fixed effects were the overall mean and the treatment, they were tested against random effects. There was no competition treatment for seedling emergence.

Source of variation	**df**	***F***	***P***	**Error term**
1 Mean	**1**	**0.24**	**0.627**	**2**
2 Species	**23**	**11.78**	**<0.001**	**5**
3 Treatment	**4**	**0.81**	**0.524**	**4**
4 Species × treatment	**91**	**1.76**	**0.002**	**5**
5 Residual	**119**			
Total	237			

Appendix B. Mean soil feedback on seedling emergence.

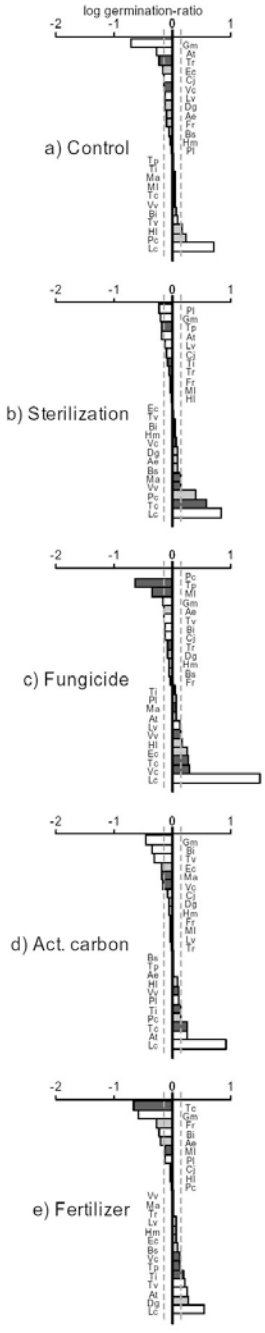

Fig. B1. Mean log-ratios for seedling emergence (mean seedling emergence probability on home soils divided by mean seedling emergence probability on away soils, log-transformed) shown separately for each species. Negative values correspond to a net disadvantage on home soils (negative feedback), positive values to a benefit on home soils (positive feedback). There was no competition treatment for seedling emergence. Dashed lines show ± 1 SEM around zero. Forbs are shown as white bars, grasses in light gray and legumes in dark gray. For species abbreviations, see Fig. 2. Pc (Fungicide): outlier, excluded.

Appendix C. Design of the main experiment.

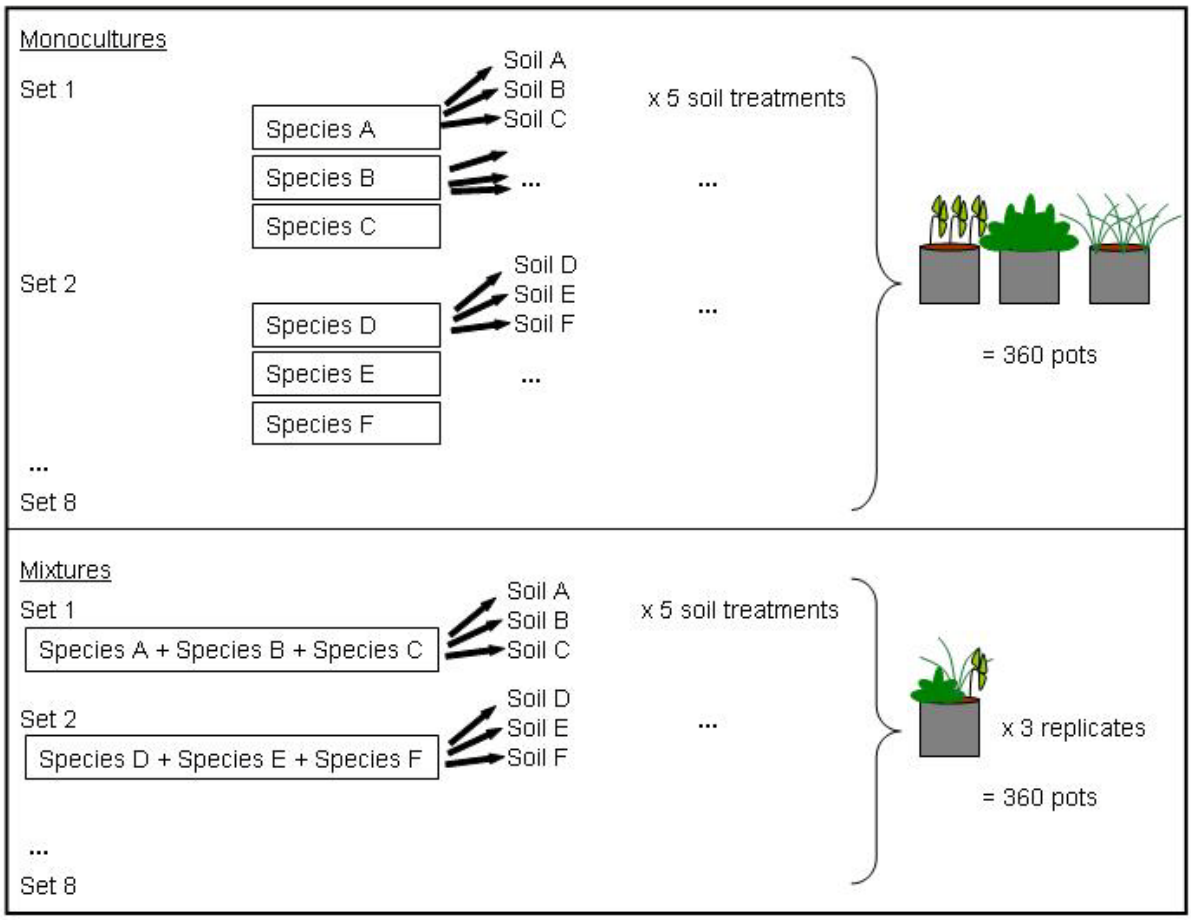

Fig. C1. Design of the main experiment: 24 species were grouped into 8 sets with one representative of each functional group (see Appendix A Table A2). For the monospecific communities ("Monocultures"), three individuals per pot were grown for each species on the three soils in its set (including its own). These 72 combinations were crossed with five soil treatments, adding up to a total of 360 pots. Multi-species communities ("Mixtures") were assembled by using one individual of each of the three species in the set per pot. This community was grown on each soil of the set, in five soil treatments. Because there were three replicates of each combination, there were also 360 mixture pots in the experiment.

Appendix D. The effect of seed dispersal on persistence time.

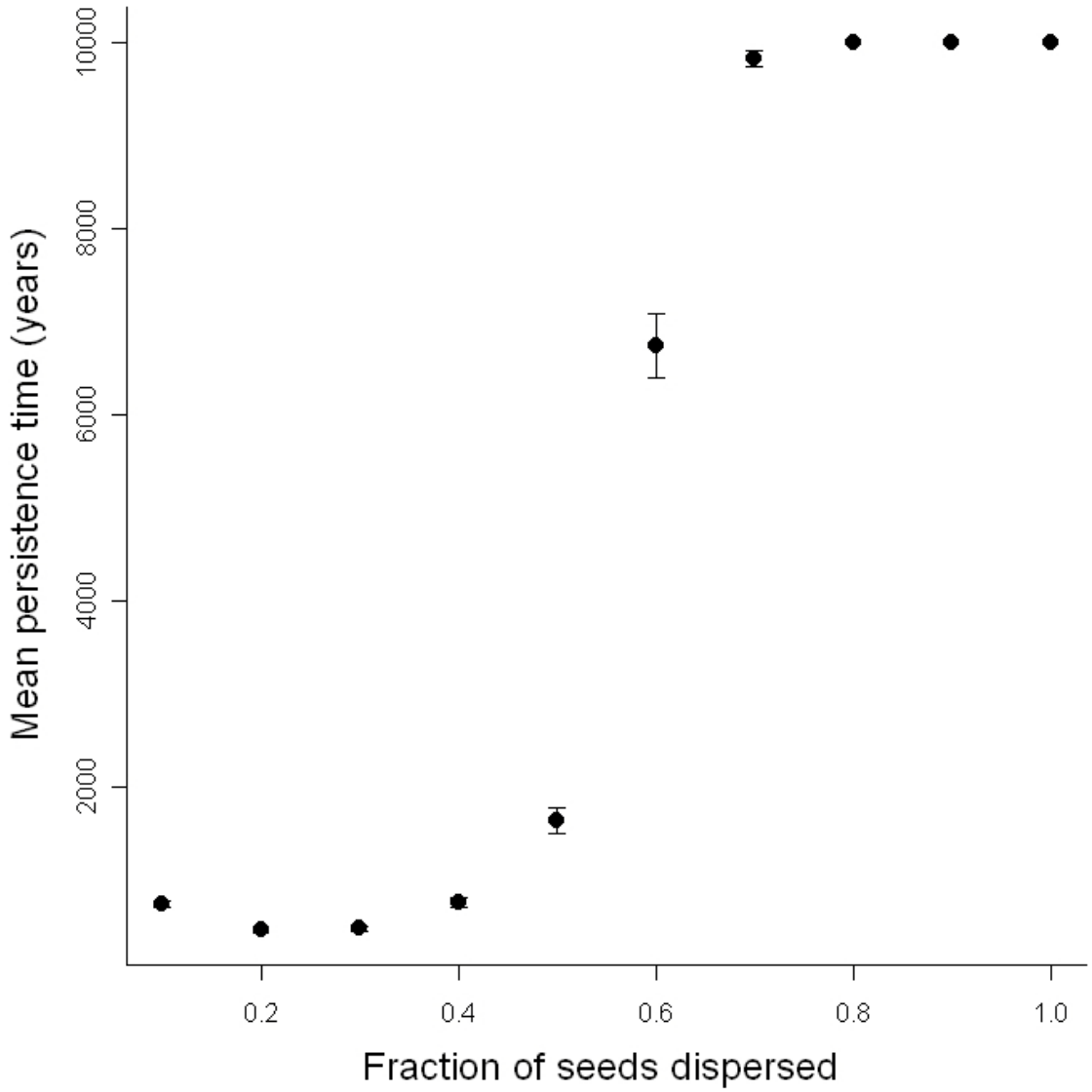

Fig. D1. The effect of increasing the fraction of seeds dispersed away from the parent site on mean persistence time. For other model parameters see text.

Chapter 5

Facilitation then negative feedbacks–sequential driving forces of community assembly in experimental grassland.

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Abstract

Plant community assembly is directed by dispersal, abiotic filtering, historical contingency, evolutionary history and biotic filtering (interactions). Here we focus on biotic filtering — to better understand species interactions and their influence on assembly. We added seeds from 48 species into established grassland communities with or without a resident species belonging to the same functional group; functional groups were forbs, grasses or legumes. We analysed immigrant seedling counts and biomass, and resident-community cover and biomass. Resident communities were either monocultures or three-species communities, and our experiment included a nutrient treatment designed to assess the role of soil fertility on biotic filtering. A phylogeny of our experimental species pool permitted additional insight into how phylogenetic relatedness between species influenced assembly outcomes.

Increasing size (aboveground cover and biomass) of resident species was the major determinant of immigrant success, reducing both the seedling number and size (aboveground biomass) of immigrant species. Diversity of residents reduced seedling numbers and biomass via increased resident-community cover and biomass, respectively. Among the mixed communities, increasing phylogenetic diversity further reduced seedling number and biomass of immigrant species via increasing resident-community biomass, suggesting that evolutionary relationships between species influence both ecosystem functioning and community assembly. Reductions of immigrant seedling numbers with nitrogen but not with phosphorus addition corresponded to increases in resident-community cover. Established immigrant species, however, increased in size with nutrient addition, grasses more in nitrogen- and legumes more in phosphorus-enriched plots. Nutrient responses suggested that the resident community dominated the uptake of added nutrients, which were therefore not available to immigrants.

Seedling counts for all three immigrant functional groups were highest in communities containing other species of the same functional group (“home” communities). This unexpected positive feedback between functionally similar species could be due to shared mutualists in the soil. For immigrant forbs and grasses, biomass was also slightly higher in home as opposed to “away” communities. Legumes,

however, produced 3–4 times the amount of biomass in away as opposed to in home communities. This negative feedback could be due to competition for phosphorus or to a build up of deleterious, legume-specific soil pathogens. In contrast to the home vs. away functional group contrast, phylogenetic distance between immigrant and resident species failed to predict immigrant performance. This suggests that in our grassland communities phylogenetic proximity was not strongly related to the functional characteristics of species relevant to resource and pathogen niches.

This paper indicates that sequential stages during immigration and thus community assembly are dominated by different biotic filters which themselves can be modified by abiotic filters such as soil nutrient availability. However, immigration and assembly processes were not related to the evolutionary history of the test species.

Introduction

Plant community assembly and subsequent composition is driven by a combination of stochastic and deterministic processes evident at different levels of community organisation (Weiher & Keddy 1995, Fukami *et al.* 2005). For this reason, disentangling and substantiating assembly rules that can predict community composition has been a difficult task, although not a fruitless one (Diamond 1975, Wilson 2007). The community assembly process is the sum of dispersal, historical contingency, abiotic filtering, biotic interactions and evolutionary history (Diaz *et al.*1998, Wilson 1999a, Ackerly 2003, Zobel *et al.* 2006, Petermann *et al.* 2010). These five constituent components of assembly control community composition, which in turn imparts community resistance to invasion and community stability under disturbance and influences ecosystem functions such as carbon storage, hydrology, nutrient cycling and productivity (Diamond 1975, Fukami & Morin 2003, Hooper 2005, Myers & Harms 2009).

Any potential immigrant must first overcome dispersal barriers and arrive at a hypothetical site (Lawton 1987, Ejrnaes *et al.* 2006). Dispersal is considered largely a stochastic process, however some species are adapted to long distance or targeted dispersal and the size and diversity of the local and regional species pool is influential (Bazzaz 1991, Hubbell 2001, Myers & Harms 2009). Correlated to dispersal differences is the sequence of species arrival, which has been shown to significantly effect

community composition (Eriksson & Eriksson 1998, Ejrnaes *et al.* 2006). This contingency of arrival sequence, if it were operating alone, could cause local communities to diverge in their composition (Diamond 1975, Drake 1991 & Fukami *et al.* 2005).

Once dispersal barriers are overcome — even if by anthropocentric causes or deliberate experimentation as in the present study — the further assembly of communities is influenced by abiotic filters and biotic interactions (Ackerly 2003, Fukami *et al.* 2005). The abiotic filter excludes immigrants unable to survive under specific local environmental conditions (Keddy 1992, Diaz *et al.*1998, Myers & Harms 2009). One particularly important abiotic factor, which we manipulated experimentally in this study, is soil fertility. The general expectation is that increased soil fertility will favour immigration of species into communities (Burke & Grime 1996, Blumenthal 2005, Davis *et al.* 2005). If abiotic filters were the only filtering processes influencing community assembly, local community compositions under common abiotic conditions would converge (Fukami *et al.* 2005, for an example see Pfisterer *et al.* 2004).

The biotic filter describes all interactions between the immigrant and the local biotic community (Lawton 1987). Therefore, in plant communities the biotic filter excludes immigrants that are unable to compete with resident plants species (Lawton 1987, Fargione 2003, Stubbs & Wilson 2004, Turnbull 2005), to resist the pressure of local pests and herbivores (above and belowground) (van der Putten 1997, Bever 2003, Petermann *et al.* 2008) or to find obligate mutualists. While herbivores, pathogens and mutualists have been shown to have a significant effect on the establishment of plant species, it is the interaction between immigrant species and the resident community that is the primary focus of this paper. This focus was selected in order to expand our understanding of the role of interspecific competition in directing community composition via biotic filtering.

Effects of species characteristics on immigration and community assembly

Early expectations of how plant species compete drew heavily on competition between sympatric animal species (Brown & Wilson 1956, Hutchinson 1959). Underlying this work were the principles of competitive exclusion (Gause *et al.* 1934, Gause 1934) and

character displacement (Brown & Wilson 1956) whereby two similar species with overlapping ranges (and comparable niche requirements) were found to differ more from each other in areas of overlap, effectively displacing one another in certain characters. Today we are more familiar with this concept as niche overlap. Limiting similarity (Macarthur & Levin 1967) was shown to relate to the range of the environment and the niche breadth of the species considered. According to successional theory (Odum 1969, Parrish and Bazzaz 1982, Bazzaz 1987, Bazzaz 1996) reduced niche overlap and niche breadth may be less strongly selected for among early- than among mid- or late-successional species because the latter should have been in closer competitive contact during their evolutionary history. For the present study we therefore used 24 early- and 24 mid-successional species (Wacker et al. 2009), assuming more complementary immigration and community assembly processes in the second than in the first group of species.

Various models incorporate limiting similarity into outcomes of coexistence and competition-colonization trade-offs (Pacala & Tilman 1994, Kinzig *et al.* 1999, Szilagyi & Meszena 2009). Recently a number of publications have addressed questions of species competition and coexistence experimentally (Fargione *et al.* 2003, Von Holle & Simberloff 2004, Turnbull *et al.* 2005, Emery 2007, Emery & Gross 2007, Mwangi *et al.* 2007, Petermann *et al.* 2008, von Felten *et al.* 2009). Taking a functional group approach, four of these studies demonstrated that established resident communities or soils trained by such communities repressed most the growth of immigrants belonging to the same functional group (Fargione *et al.* 2003, Turnbull *et al.* 2005, Mwangi *et al.* 2007, Petermann *et al.* 2008). The four remaining studies found little or no support for limiting similarity on a functional group level (Von Holle & Simberloff 2004, Emery 2007, Emery & Gross 2007, von Felten *et al.* 2009). To test whether immigration is a functional-group complementary process (Petermann *et al.* 2010); we included three functional groups in our test-plant communities, i.e. grasses, forbs and legumes.

When employing functional groups or guilds, the intention is to combine species on the foundation of ecological as opposed to taxonomic similarities (Wilson 1999b). Such groupings are, however, generally crude representations of the ecology of a species, and may be no better than taxonomic groups in predicting niche overlap. The

functional group approach can be extended by adopting a more precise measure of evolutionary relatedness. Evolutionary relationships between two species can be informative with respect to the species ecological function in a community if they have evolved separately and maintained their niche (phylogenetic niche conservatism) but not if their niches converged under common selection pressures, such as in a shared environment (Webb *et al.* 2002, Cavender-Bares *et al.* 2004). If niches are conserved, then competitive interactions between species will result in community phylogenetic overdispersion — co-occurring species are more distantly related than randomly expected (Cavender-Bares *et al.* 2004, Emerson & Gillespie 2008). Analysis of trait variation among species has demonstrated niche conservatism (Prinzing *et al.* 2001), however, currently available empirical results suggest that the intensity of interspecific competition is only weakly linked to the degree of species relatedness (Lambdon & Hulme 2006, Cahill *et al.* 2008, Cadotte *et al.* 2009). To test whether immigration is a phylogenetically complementary process, we built a phylogeny for our 48 test species and related immigrant success to phylogenetic distance between immigrant and resident species.

Nutrients and community assembly

Drawing on results from invasion ecology (Blumenthal 2005, Davis *et al.* 2000), soil nutrient availability can be expected to influence community assembly. For example, Burke and Grime (1996) found that eutrophication in tandem with disturbance can increase community susceptibility to invasion (Burke & Grime 1996). Nitrogen enrichment, both by native or exotic species, has also been shown to facilitate, respectively, initial or additional invasive species (Scherer-Lorenzen *et al.* 2007). In each of our experimental communities nitrogen, phosphorous, or both nitrogen and phosphorous, were applied to quadrats in order to assess the role of soil fertility on biotic filtering.

Approach and hypotheses

In order to understand the role of competition in directing community assembly and composition we added seeds of our 24 early- and 24 mid-successional species to

dissimilar, simplified early and mid-successional communities and crossed the corresponding treatments with different nutrient addition treatments. To test if reciprocal immigration at the functional group level is more likely than self-replacement we added the seeds of the species to resident communities where immigrants were either present or absent at the functional group level. The three functional groups we distinguished were grasses, forbs and legumes, these groupings were also used in a number of previous biodiversity experiments (Hector *et al.* 1999, Niklaus *et al.* 2001, Wacker *et al.* 2008). We followed the germination (seedling counts) and growth (aboveground biomass) of immigrant species, while also measuring the development of the resident plant cover and aboveground biomass. Our expectation was that more immigrant seedlings and more immigrant biomass would be found in communities where the immigrant species was functionally absent (hypothesis 1), and that this complementarity between immigrant and resident species would be stronger in mid- than in early-successional communities (hypothesis 2). Immigrants should perform better in “away” plots because species can coexist by avoiding resource-niche overlap or the accumulation of soil pathogens. Resource acquisition traits may be phylogenetically conserved and pathogens may be host-conserved, thus both are related to phylogenetic distance between immigrants and residents. We expected immigrant species to perform better in communities where the resident species were more distantly related (hypothesis 3), as we assumed phylogenetic niche conservatism for our species. For mixed resident communities we expected increasing phylogenetic diversity to increase community immigration resistance (hypothesis 4), most likely via increased resident-community biomass. In all plots we assessed the influence of soil nutrient availability on community assembly processes. We hypothesised that immigrant species would be able to exploit increased soil fertility and there would be more seedlings and increased biomass of immigrants in quadrats with added nutrients (hypothesis 5). However, increased nutrient availability should increase resource competition between immigrant and resident species belonging to the same functional group, leading us to expect reduced immigrant germination and growth in home plots with added nutrients (hypothesis 6).

Material & Methods

Experimental design

Our experiment was conducted at the agricultural extension station Forschungsanstalt Agroscope Reckenholz-Tänikon ART in Zürich, Switzerland. This site has a sandy-loamy soil with a pH of 7.6±0.2, a mean concentration of soluble nitrogen of 26±0.9 mg kg-1 and a mean concentration of soluble phosphorus of 4±0.3 mg kg-1. Prior to our experiment the field was planted with crop species. At the beginning of the experiment in April 2003 the field was harrowed. Our assembly experiment was nested within a larger biodiversity experiment, of which further details are available in Wacker *et al.* (2009). We selected 48 herbaceous plant species, all of which occur commonly in central-European grasslands. These 48 species were separated into eight non-overlapping species pools each containing six species (Table A1). Four of these species pools were made up of early-successional species, and four were made up of mid-successional species. The successional break was defined using life history traits of species identified in the literature (Lauber & Wagner 2007). The six species in each pool represented the three major functional groups found in central-European grasslands: forbs, grasses and legumes, and in roughly the same proportion as they would be found in a natural system: three forb to two grass to one legume species. Each of the eight non-overlapping species pools was split further into two three-species sub-pools; species were placed into each sub-pool by random splitting of the total pool (Table A1). From each pool eight experimental communities were established: monocultures for each of the six species and two non-overlapping three-species communities reflecting the sub-pools (Table A1). The monoculture plots were not replicated and yielded 6 x 8 = 48 plots. The three-species communities were replicated once, adding 2 x 8 x 2 = 32 further plots, resulting in a total of 80 plots.

Seed and nutrient addition

At the start of our experiment, in April 2005, the experimental resident communities had been growing for 2 full years on the 80 plots. Each of the 80 plots was now dissected into a core area (50 x 200 cm) and 8 strips (25 x 100 cm), and each strip was subdivided into 4 quadrats (25 x 25 cm) (Fig. A1). On the plot level the diversity and

composition/identity (three-species communities/monocultures) of resident communities varied. The immigrant species treatment was applied to the strip level and the nutrient treatment was applied to quadrats (Fig. A1). The immigrant species that were added to the strips within each plot belonged to the same pool as the resident species, so each plot always contained resident and immigrant species from the same pool. In each of six strips we sowed a different single species from the six-species pools; the seventh strip received an additional legume species to balance the design in terms of the number of functional groups being added as immigrant species. This resulted in a mechanistic diallel (McGilchrist 1965) of interspecific interactions (Fig. A2). The eighth strip in each plot received no seed addition and served as a control. Because species of more than one functional group were commonly present in the three-species communities, the number of tests of immigrants entering communities where they were functionally absent (away tests) was smaller than for monocultures. In the three-species communities for three strips we could not distinguish immigrant from resident species, as the immigrant being added was the same species as one of the residents. For the monoculture communities this was less of a problem, as only in one strip was the immigrant the same species as the resident.

The original intention had been to follow labelled seedlings in quadrats where the immigrant and resident were the same species, but this proved too difficult. Seeds were added to resident communities in April 2005 at a constant density (1000 viable seeds m-2). Viability of seeds had previously been measured with germination trials. At the time of sowing, two of the species from the original design and experimental setup were unavailable; therefore strips which should have received *Diplotaxis tenuifolia* or *Lepidium virginicum* remained unseeded, and total immigrant number was reduced to 46 species.

From the beginning of the experiment in 2003, nutrients were added across each plot in a chequerboard pattern at the 25 x 25 cm quadrat scale (Wacker *et al.* 2008, Wacker *et al.* 2009). Quadrats received one of four different nutrient additions as granular fertilizer (AGROline AG, Basel, Switzerland): (1) no fertilizer (control), (2) nitrogen, (3) phosphorus, (4) nitrogen and phosphorus (Fig. A1). The size of the quadrats selected for nutrient addition had been assessed in a previous pilot

experiment, where it proved to be adequate for the species used in our experiment. Subsequent growth of vegetation revealed nutrient application to be very precise, with no evidence of lateral movement of nutrients. Nitrogen was added twice per year at 8 g/m². Phosphorus was added twice per year at 4 g/m². The quadrats where both nitrogen and phosphorus were added received 4 g/m² and 2 g/m² twice per year.

Measurements

Total cover of resident species was recorded for each quadrat (0.25 x 0.25 m) in April 2005, at the time of seed addition, and again in May and July. Sown seedling numbers were recorded in the seven sown strips and in the associated unsown control strip in May and again in July. An unsuccessful attempt was made to mark a number of seedlings and follow their growth and survival, so to have seedling counts in quadrats where the immigrant and resident were the same species. From 20–26 June and again from 29 July to 4 August all aboveground biomass was harvested in each subplot at the quadrat level at a height of 5 cm above the soil surface. Biomass was sorted into species and dried at 80°C for 48 hours and subsequently weighed. In strips where the immigrant species that was added was the same as the resident species, we could not distinguish between the relative contributions of the original resident, offspring from the resident or the immigrant plants.

Molecular Phylogeny

For each of the 48 species, we searched Genbank for 4 gene sequences (a mix of coding and non-coding genes) commonly used in published angiosperm phylogenies: its1, matk, rbcl, and 5.8s (Cadotte 2008). Of our 48 species, 39 species had at least one gene represented in Genbank, with the majority of species having two or three genes represented (Benson *et al* 2005). For nine species, we used gene sequences for a congeneric relative. In addition, we included two representatives of early diverging lineages as outgroup species; these were *Amborella trichopoda* Baill. and *Magnolia grandiflora* L. For these 50 species we aligned sequences using the program MUSCLE (Edgar 2004). We then selected best-fit models of nucleotide substitution for each gene using the Akaike Information Criterion (AIC), as implemented in the program Modeltest

(Posada & Crandall 1998). From the gene sequences a maximum likelihood phylogeny was composed. Using the aligned sequences, the best-fit models of nucleotide substitution were used to estimate a maximum likelihood phylogeny using the PHYML algorithm (Fig. A3) (Anisimova & Gascuel 2006). To assess nodal support on maximum likelihood phylogenies, we report approximate Likelihood-Ratio Test (aLRT) scores (Fig. A3) (Guindin & Gascuel 2003).

From the phylogeny, phylogenetic distance between species was calculated by summing branch lengths between species. Given our phylogeny of 50 species, the phylogenetic diversity of each three-species communities was the sum of the branch lengths of a minimal subtree connecting the three-species. To calculate phylogenetic diversity we used code provided by T. Jonathan Davies that was run in R (version 2.6.2, R Development Core Team 2009) (Cadotte 2008). This method is similar to others such as Faith's phylogenetic diversity, a metric identical to ours except that it calculates diversity with the inclusion of the root node of a larger regional phylogeny (Faith 1992). Faith's PD then is a measure of the proportion of evolutionary history represented within a local community. Our measure of PD calculates the phylogenetic distance connecting all members of a community together without considering a larger regional phylogeny.

Statistical analysis

All statistical analyses were completed with the statistical software R (version 2.10.1, R Development Core Team 2009). Immigrant seedling counts from May and July were averaged. Immigrant biomass for the June and August harvests was summed, as was resident biomass from the June and August harvests. Summed immigrant biomass and the covariate summed resident biomass were both logged to obtain normality and homoscedasticity of residuals. Mean immigrant seedling count and summed immigrant biomass were analysed using generalized and general linear mixed-effects models, respectively, employing the LMER function from the lme4 library (Bates & Maechler 2009). LMER was selected as it allowed for appropriate treatment of the error structure of the experiment and for the crossed random factors. The random error structure differed between models including both monocultures and three-species communities, and those just focusing on the monocultures. For all monoculture models, block, pool,

resident species, immigrant species, and the interaction between resident and immigrant species were considered random effects. Because the three-species communities had three resident species, yet a single immigrant species, models including residents and the interaction between residents and immigrants as random effects could not be run by LMER, so for these models, plot and the plot x immigrant interaction were used instead as random effects against which fixed effects could be tested.

There was a similar difference between the monoculture and mixture models regarding fixed effects. For the monoculture models a single fixed-effects factor of resident functional group was included in the model. Because this was not possible for the three-species communities, three factors representing each functional group were used instead, forb, grass, and legume. The remaining fixed effects included successional status, functional group of the immigrant, nutrient addition, and diversity for those models including both community types. For all models a full model was run first, followed by stepwise removal of non-significant interactions using AIC values to end with an optimal model (Johnson & Omland 2004). Summary tables from the LMER model permitted extraction of variance components for each of the random effects. Probability estimates of the fixed effects were calculated using Markov Chain Monte Carlo (MCMC) simulations. As each model had at minimum 4 fixed effects and multiple interactions, calculation of uniquely defined predicted values for particular treatment combinations was difficult. For this reason, we present means and standard errors of the raw data instead and support comparisons between particular treatment combinations with significance tests from the analyses. Parameter estimates for each model, however, are available in the appendix.

To visualise the functional group home/away and family home/away responses, ratios of immigrant seedling numbers and biomass when grown in home and away communities were calculated. For seedlings, the total number of seedlings grown in communities with residents of the same functional group was divided by the total number of all individuals of that species grown in communities where the residents belonged to other functional groups, generating a proportional measure of immigrant success. For biomass, the aboveground biomass of individuals grown in communities

with residents of the same functional group was divided by the aboveground biomass of all individuals of that species grown in communities where the residents belonged to other functional groups, generating a second proportional measure of immigrant success. For two species we could not calculate biomass ratios as they produced no biomass in home communities. For these two species, *Phleum pratense* L., and *Rumex acetosella* L., we added the next smallest value of average biomass in home communities (0.01 g). A single species also produced no biomass on away plots, *Lepidium campestre* (L.) W.T.Aiton. In this case, we added the next smallest value from the away communities (0.03 g). The ratio was log-transformed to achieve normality and homogeneity of variances. At the same time, the log transformation returns zero when there is no difference between immigrant performance in home and away communities, and positive values where immigrants perform better in home communities. A second set of logged home/away ratios were calculated using a family home/away comparison. For nine species, there were no seedlings in away communities using the family comparison; in these cases the lowest number of seedlings evident from the other species was used as a surrogate (1).

Results

Resident-community cover and biomass

Analyses of resident cover and resident biomass were completed for monocultures and three-species communities together (both community types), and also for monocultures alone (monocultures). The successional status of the species comprising the resident community was the most important factor determining community cover, for analyses of both community types (Table 1, $F_{1,1592} = 57.46$, $P<0.0001$) and for monocultures (Table 2, $F_{1,1069} = 28.15$, $P<0.0001$). On average mid-successional communities had a cover score of 2.74±0.03 (mean±se of both community types) of a potential 4 (100% cover) versus a score of 1.17±0.04 for early-successional species. The increase in diversity from monocultures to three-species communities significantly increased average resident cover from a score of 1.57±0.04 in monocultures to a score of 2.53±0.04 in three-species communities (Table 1, $F_{1,1592} = 18.83$, $P<0.0001$).

Table 1. Variance components and a fixed effects ANOVA for a mixed effects model of resident cover measured in April 2005, for both monocultures and three-species communities. Statistical significance of fixed effects was determined using Markov chain Monte Carlo simulations to calculate probability estimates. The terms grass, forb and legume refer to the presence of each of these functional groups in the resident community (either in monoculture or mixture). Fixed effects that are significant at the 5 % threshold are shown in italics. Analysed with R (version 2.10.1).

Variance components for random terms	Variance	SD
block	0.0000	0.0000
pool	0.0000	0.0000
plot (resident species)	0.7809	0.8837
strip (resident species x immigrant species)	0.0925	0.3041
residual	0.5257	0.7251

Fixed effects	DF	SS	MS	F	DF2	P
immigrant biomass	*1*	*6.0856*	*6.0856*	*11.5758*	*1592*	*0.0007*
diversity	*1*	*9.8991*	*9.8991*	*18.8297*	*1592*	*<0.0001*
succession	*1*	*30.2100*	*30.2100*	*57.4644*	*1592*	*<0.0001*
forb	1	1.3434	1.3434	2.5553	1592	0.1101
grass	1	1.6262	1.6262	3.0933	1592	0.0788
legume	1	0.5556	0.5556	1.0568	1592	0.3041
functional immigrant	2	2.7948	1.3974	2.6581	1592	0.0704
nutrient	3	3.8954	1.2985	2.4699	1592	0.0603

The addition of nutrients was the single most important factor significantly explaining resident biomass, for analyses of both community types (Table 3, $F_{3,1548}$ = 53.79, P < 0.0001) and monocultures (Table 4, $F_{3, 1036}$ = 34.53, P < 0.0001). Resident communities responded to nutrient addition with biomass increases in quadrats with additional nitrogen, but with little change in plots with phosphorous addition (Fig 1.A). Quadrats that received both nitrogen and phosphorous increased most to an average of 49.68±1.68 g per 0.25 x 0.25 m quadrat compared to the control quadrat at 32.92±1.17 g per 0.25 x 0.25 m (Fig. 7A). Averaged over both community types, mid-successional resident communities produced almost twice as much biomass — 54.11 ±1.02 g per 0.25 x 0.25 m quadrat — as early-successional resident communities, 28.73±0.97 g (Table 3, $F_{1,1548}$ = 35.80, P < 0.0001). The analysis of monocultures alone was consistent with this trend, mid-successional resident monocultures produced 43.84±1.23 g biomass per 0.25 x 0.25 m quadrat and early-successional monocultures produced 18.99±0.92 g biomass per 0.25 x 0.25 m quadrat (Table 4, $F_{1,1036}$ = 24.24, P < 0.0001).

Table 2. Variance components and a fixed effect ANOVA for a mixed effects model of resident cover measured in April 2005, for monocultures only. Statistical significance of fixed effects was determined using Markov chain Monte Carlo simulations to calculate probability estimates. Fixed effects that are significant at the 5 % threshold are shown in italics. Analysed with R (version 2.10.1).

Variance components for random terms	Variance	SD
Block	0.0518	0.2276
Pool	0.0000	0.0000
resident species	0.8985	0.9479
immigrant species	0.0000	0.0000
resident species x immigrant species	0.0807	0.2841
Residual	0.5264	0.7256

Fixed effects	DF	SS	MS	F	DF2	P
immigrant biomass	1	2.9951	2.9951	5.6895	1069	0.0172
functional resident	2	1.2287	0.6144	1.1670	1069	0.3117
succession	1	14.8208	14.8208	28.1541	1069	0.0000
functional immigrant	2	2.2351	1.1175	2.1229	1069	0.1202
nutrient	3	2.8378	0.9459	1.7969	1069	0.1460
immigrant biomass x functional immigrant	2	0.4814	0.2407	0.4573	1069	0.6331
functional resident x functional immigrant	4	3.5144	0.8786	1.6690	1069	0.1549
succession x functional immigrant	2	1.8982	0.9491	1.8030	1069	0.1653
immigrant biomass x nutrient	3	1.9536	0.6512	1.2370	1069	0.2950
functional resident x nutrient	6	2.1344	0.3557	0.6758	1069	0.6693
succession x nutrient	3	0.1524	0.0508	0.0965	1069	0.9620
functional immigrant x nutrient	6	2.0923	0.3487	0.6624	1069	0.6801
immigrant biomass x functional resident	2	0.7023	0.3511	0.6671	1069	0.5134
immigrant biomass x succession	1	0.5958	0.5958	1.1318	1069	0.2876
functional resident x succession	2	0.9026	0.4513	0.8573	1069	0.4246
immigrant biomass x succession x functional immigrant	2	4.3885	2.1943	4.1683	1069	0.0157

All four mid-successional pools of resident species outperformed the early-successional pools (pool was a random term in both models, Tables 3 & 4). The increase in diversity between monoculture and three-species mixture communities, resulted in a significant increase of resident biomass from 31.42±0.83 g biomass per 0.25 x 0.25 m quadrat in monocultures to 56.43±1.35 g in three-species communities (Fig. 2A & Table 3, $F_{1,1548} = 19.67$, $P < 0.0001$). Analysis of other diversity metrics was completed for the three-species communities. Increasing functional group diversity (ranging from 1–3 functional groups) resulted in increased resident community biomass (Fig. 2B & Table A16, $F_{2, 943} = 6.90$, $P = 0.0011$). Similarly, three-species communities with higher phylogenetic diversity (ranging from 0.42 and 0.86 PD units) among also produced on average more resident-community biomass ($F_{1, 195} = 9.17$, $P < 0.0028$).

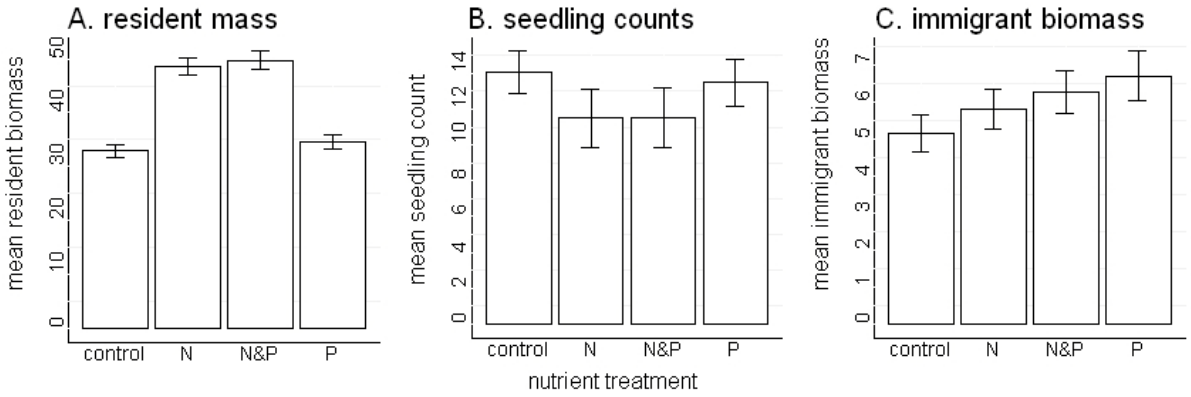

Figure 1. Mean responses to nutrient addition per 0.25 x 0.25 m quadrat: mean resident biomass (A), mean immigrant seedling number (B), and mean immigrant biomass (C) each against four nutrient treatments. Nutrient treatments: control; N = nitrogen; N&P = nitrogen and phosphorous; P = phosphorous. Nutrient addition quantity: nitrogen = 2 x 8 g/m² (annually); phosphorous = 2 x 4 g/m² (annually); nitrogen and phosphorous = 2 x 4 g/m² N and 2 g/m² P (annually). Error bars = standard error of the mean.

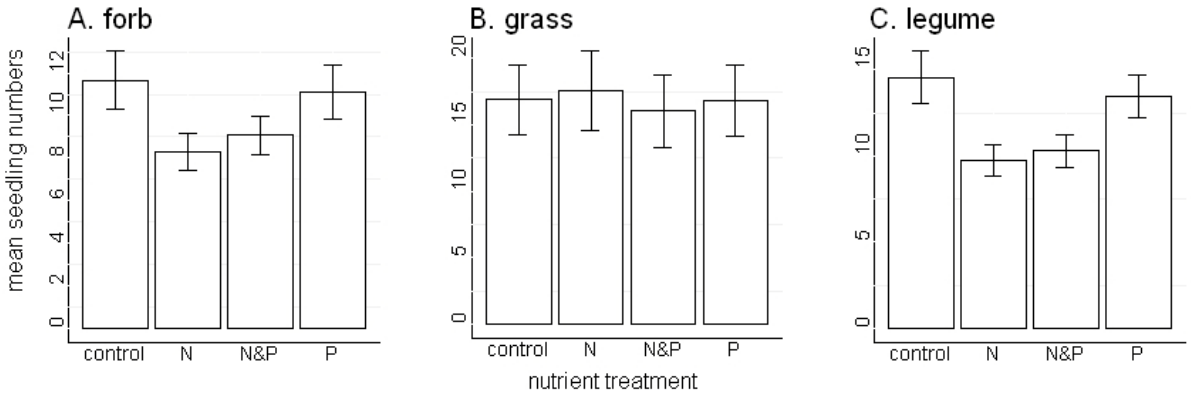

Figure 2. Mean seedling numbers per 0.25 x 0.25 m quadrat of each functional group in response to nutrient addition: forb (A), grass (B), and legume(C). Nutrient treatments: control; N = nitrogen; N&P = nitrogen and phosphorous; P = phosphorous. Nutrient addition quantity: nitrogen = 2 x 8 g/m² (annually); phosphorous = 2 x 4 g/m² (annually); nitrogen and phosphorous = 2 x 4 g/m² N and 2 g/m² P (annually). Error bars = standard error of the mean.

Table 3. Variance components and a fixed effect ANOVA for a mixed effects model of mean resident biomass (averaged over two harvests) for both monocultures and three-species communities. Statistical significance of fixed effects was determined using Markov chain Monte Carlo simulations to calculate probability estimates. The terms grass, forb and legume refer to the presence of each of these functional groups in the resident community (either in monoculture or mixture). Fixed effects that are significant at the 5 % threshold are shown in italics. Analysed with R (version 2.10.1).

Variance components for random terms	Variance	SD
block	0.0040	0.0631
pool	0.0001	0.0099
plot (resident species)	0.7485	0.8651
strip (resident species x immigrant species)	0.2193	0.4683
residual	0.5907	0.7686

Fixed effects	DF	SS	MS	F	DF2	P
immigrant biomass	*1*	*12.839*	*12.839*	*21.7346*	*1548*	*<0.0001*
diversity	*1*	*11.618*	*11.618*	*19.6677*	*1548*	*<0.0001*
succession	*1*	*21.147*	*21.147*	*35.7996*	*1548*	*<0.0001*
forb	1	0.637	0.637	1.0785	1548	0.2992
grass	1	1.727	1.727	2.9245	1548	0.0875
legume	1	0.105	0.105	0.1778	1548	0.6734
functional immigrant	2	1.931	0.966	1.6345	1548	0.1954
nutrient	*3*	*95.315*	*31.772*	*53.7860*	*1548*	*<0.0001*
immigrant biomass x functional immigrant	*2*	*7.628*	*3.814*	*6.4567*	*1548*	*0.0016*
immigrant biomass x nutrient	3	3.990	1.330	2.2518	1548	0.0806
immigrant biomass x succession	*1*	*4.055*	*4.055*	*6.8642*	*1548*	*0.0089*
grass x functional immigrant	*2*	*5.725*	*2.863*	*4.8463*	*1548*	*0.0080*
legume x functional immigrant	*2*	*5.006*	*2.503*	*4.2371*	*1548*	*0.0146*
legume x nutrient	*3*	*5.779*	*1.926*	*3.2609*	*1548*	*0.0208*

Table 4. Variance components and a fixed effect ANOVA for a mixed effects model of mean resident biomass (averaged over two harvests) for monocultures only. Statistical significance of fixed effects was determined using Markov chain Monte Carlo simulations to calculate probability estimates. Fixed effects that are significant at the 5 % threshold are shown in italics. Analysed with R (version 2.10.1).

Variance components for random terms	Variance	SD
block	0.0000	0.0000
pool	0.0000	0.0000
resident species	0.9055	0.9516
immigrant species	0.0000	0.0000
resident species x immigrant species	0.2778	0.5271
residual	0.7160	0.8462

Fixed effects	DF	SS	MS	F	DF2	P
immigrant biomass	*1*	*8.6890*	*8.6889*	*12.1355*	*1036*	*0.0005*
functional resident	2	0.6420	0.3210	0.4484	1036	0.6388
succession	*1*	*17.3560*	*17.3559*	*24.2404*	*1036*	*0.0000*
functional immigrant	2	3.2850	1.6424	2.2939	1036	0.1014
nutrient	*3*	*74.1810*	*24.7269*	*34.5353*	*1036*	*0.0000*
immigrant biomass x functional immigrant	*2*	*5.8990*	*2.9496*	*4.1196*	*1036*	*0.0165*
functional resident x functional immigrant	*4*	*9.3170*	*2.3292*	*3.2532*	*1036*	*0.0116*
succession x functional immigrant	2	0.4820	0.2409	0.3364	1036	0.7144
immigrant biomass x nutrient	3	4.4690	1.4896	2.0804	1036	0.1011
functional resident x nutrient	6	6.8010	1.1336	1.5832	1036	0.1486
succession x nutrient	*3*	*5.6320*	*1.8773*	*2.6220*	*1036*	*0.0494*
functional immigrant x nutrient	6	4.2660	0.7110	0.9930	1036	0.4286
immigrant biomass x functional resident	2	3.6810	1.8403	2.5703	1036	0.0770
immigrant biomass x succession	*1*	*5.8500*	*5.8500*	*8.1705*	*1036*	*0.0043*
functional resident x succession	2	0.4000	0.1999	0.2791	1036	0.7565
immigrant biomass x functional resident x functional immigrant	*4*	*8.2240*	*2.0561*	*2.8716*	*1036*	*0.0221*

Effects of immigrant species characteristics on immigration

Analysis of both community types showed nutrient addition to significantly influence seedling counts (Table 5, $F_{3,1574} = 9.17$, $P < 0.0001$), seedling numbers were reduced in all quadrats receiving nutrient addition (Fig. 1B). Analysis of the monocultures was similar (Table 6, $F_{3, 986} = 4.64$, $P = 0.0031$). On average, in communities receiving additional nitrogen, seedling numbers were reduced most from 14.06±1.17 per 0.25 x 0.25 m control quadrats to 11.49±1.63 and 11.50±1.68 seedlings per 0.25 x 0.25 m in the nitrogen addition and the combined nitrogen and phosphorous addition quadrats respectively (Fig. 1B). In quadrats receiving only phosphorous addition there was a small reduction in immigrant seedling numbers to 13.48±1.3 per

0.25 x 0.25 m. The analysis of both community types for seedling counts revealed a significant two way interaction between the functional group of the immigrant and the nutrient addition treatment (Table 5, $F_{6,1574} = 5.37$, $P < 0.0001$). This interaction signifies that seedlings of the immigrant functional groups responded differently to nutrient addition, decreases in the nitrogen treatments were forb and legume seedling driven responses (Fig. 2A & 2C) while grass seedlings did not significantly respond to any of the nutrient treatments (Fig. 2B). The monoculture analysis indicated the same trend (Table 6, $F_{6,986} = 4.56$, $P = 0.0001$).

The latter measure of immigrant success, immigrant biomass, exhibited another pattern in response to nutrient addition (Fig. 1C), albeit non-significantly. In all three quadrats receiving nutrient addition, the average biomass of immigrants across the experiment increased, however it was in plots enriched with phosphorous where this was most evident, with the greatest increase being between the control quadrats (5.17±0.49 g per 0.25 x 0.25 m) and the quadrats receiving only phosphorous addition (6.72±0.68 g per 0.25 x 0.25 m). As with the seedling counts, the analysis of immigrant biomass for both community types identified a significant two way interaction between the functional group of the immigrant and the nutrient addition treatment (Table 7, $F_{6,924} = 2.75$, $P < 0.0117$). This interaction signifies the summed size of the different immigrant functional groups responded differently to nutrient addition (Fig. 3). Immigrant forb species did not respond to the nutrient addition, except for a minor increase in the combined nitrogen and phosphorous addition quadrats (Fig. 3A). Immigrant grass species increased their biomass to more than double that found in the control quadrats when nitrogen alone was added to quadrats (Fig. 3B). With increasing phosphorus addition grass immigrant biomass decreased consistently from its peak in the nitrogen addition quadrat (Fig. 3B). Immigrant legume species responded in the opposite direction to grasses, immigrant legume biomass decreased slightly from the control to nitrogen quadrats, and then increased in steps with increasing phosphorus addition (Fig. 3C). There was not a significant interaction between the functional group of the immigrant and the nutrient addition treatment for the monoculture analysis.

Table 5. Variance components and a fixed effect ANOVA for a mixed effects model of mean seedling counts (summed over two counts), for both monocultures and three-species communities. Statistical significance of fixed effects was determined using Markov chain Monte Carlo simulations to calculate probability estimates. The terms grass, forb and legume refer to the presence of each of these functional groups in the resident community (either in monoculture or mixture). Fixed effects that are significant at the 5 % threshold are shown in italics. Analysed with R (version 2.10.1).

Variance components for random terms	Variance	SD
block	0.0000	0.0000
pool	0.0000	0.0000
plot (resident species)	0.0318	0.1782
immigrant species	0.3999	0.6324
strip (resident species x immigrant species)	0.5886	0.7672
residual	0.3414	0.5843

Table 5. continued.

Fixed effects	DF	SS	MS	F	DF2	P
cover april	*1*	*33.449*	*33.449*	*97.9719*	*1574*	*<0.0001*
diversity	1	0.146	0.146	0.4283	1574	0.5129
succession	1	0.001	0.001	0.0028	1574	0.9578
grass	1	0.008	0.008	0.0222	1574	0.8815
forb	1	0.099	0.099	0.2901	1574	0.5903
legume	1	0.883	0.883	2.5875	1574	0.1079
functional immigrant	2	0.404	0.202	0.5915	1574	0.5536
home/away	*1*	*11.298*	*11.298*	*33.0936*	*1574*	*<0.0001*
nutrient	*3*	*9.387*	*3.129*	*9.1650*	*1574*	*<0.0001*
cover april x diversity	*1*	*3.827*	*3.827*	*11.2087*	*1574*	*0.0008*
cover april x succession	1	0.607	0.607	1.7777	1574	0.1826
diversity x succession	1	0.007	0.007	0.0198	1574	0.8881
cover april x grass	1	0.493	0.493	1.4430	1574	0.2298
succession x grass	1	0.772	0.772	2.2602	1574	0.1329
cover april x forb	1	0.000	0.000	0.0000	1574	0.9990
succession x forb	1	0.191	0.191	0.5599	1574	0.4544
cover april x legume	1	0.999	0.999	2.9270	1574	0.0873
succession x legume	1	0.018	0.018	0.0528	1574	0.8183
grass x home/away	1	0.076	0.076	0.2216	1574	0.6379
grass x nutrient	3	0.548	0.183	0.5351	1574	0.6582
forb x home/away	1	0.052	0.052	0.1530	1574	0.6958
forb x nutrient	3	2.204	0.735	2.1520	1574	0.0919
legume x nutrient	3	0.554	0.185	0.5405	1574	0.6546
cover april x functional immigrant	*2*	*2.507*	*1.253*	*3.6713*	*1574*	*0.0257*
diversity x functional immigrant	2	1.919	0.960	2.8109	1574	0.0605
succession x functional immigrant	*2*	*3.695*	*1.847*	*5.4108*	*1574*	*0.0046*
cover april x home/away	1	0.007	0.007	0.0202	1574	0.8871
diversity x home/away	*1*	*2.315*	*2.315*	*6.7804*	*1574*	*0.0093*
succession x home/away	1	0.052	0.052	0.1514	1574	0.6972
cover april x nutrient	3	2.255	0.752	2.2018	1574	0.0860
diversity x nutrient	3	1.051	0.350	1.0261	1574	0.3800
succession x nutrient	*3*	*3.581*	*1.194*	*3.4961*	*1574*	*0.0151*
functional immigrant x nutrient	*6*	*10.995*	*1.833*	*5.3677*	*1574*	*<0.0001*
home/away x nutrient	3	1.789	0.596	1.7470	1574	0.1554
functional immigrant x home/away	2	1.577	0.788	2.3090	1574	0.0997
cover april x diversity x succession	*1*	*2.066*	*2.066*	*6.0527*	*1574*	*0.0140*
cover april x diversity x functional immigrant	2	1.299	0.650	1.9030	1574	0.1495
cover april x succession x nutrient	3	1.598	0.533	1.5603	1574	0.1972
cover april x succession x grass	1	0.864	0.864	2.5309	1574	0.1118
cover april x succession x forb	1	0.962	0.962	2.8176	1574	0.0934
diversity x succession x nutrient	*3*	*3.500*	*1.167*	*3.4168*	*1574*	*0.0168*

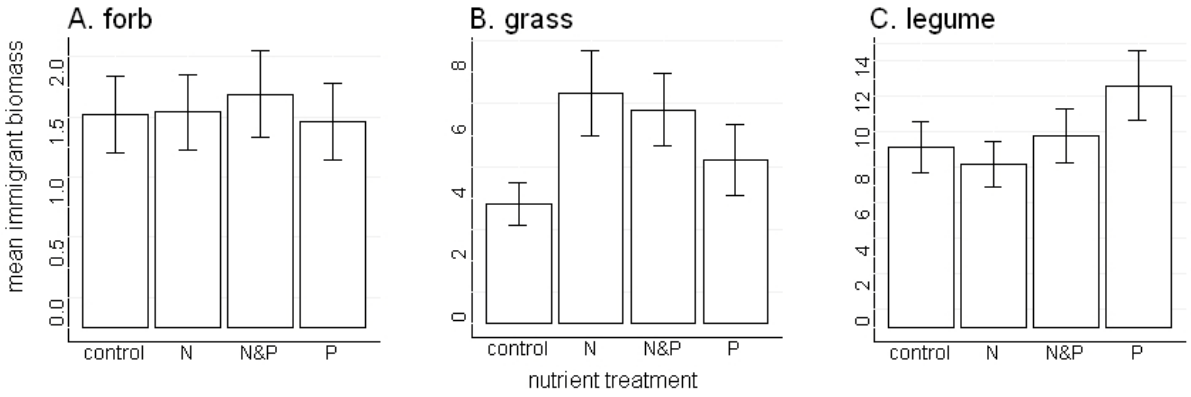

Figure 3. Mean immigrant biomass per 0.25 x 0.25 m quadrat of each functional group in response to nutrient addition: forb (A), grass (B), and legume(C). Nutrient treatments: control; N = nitrogen; N&P = nitrogen and phosphorous; P = phosphorous. Nutrient addition quantity: nitrogen = 2 x 8 g/m² (annually); phosphorous = 2 x 4 g/m² (annually); nitrogen and phosphorous = 2 x 4 g/m² N and 2 g/m² P (annually). Error bars = standard error of the mean.

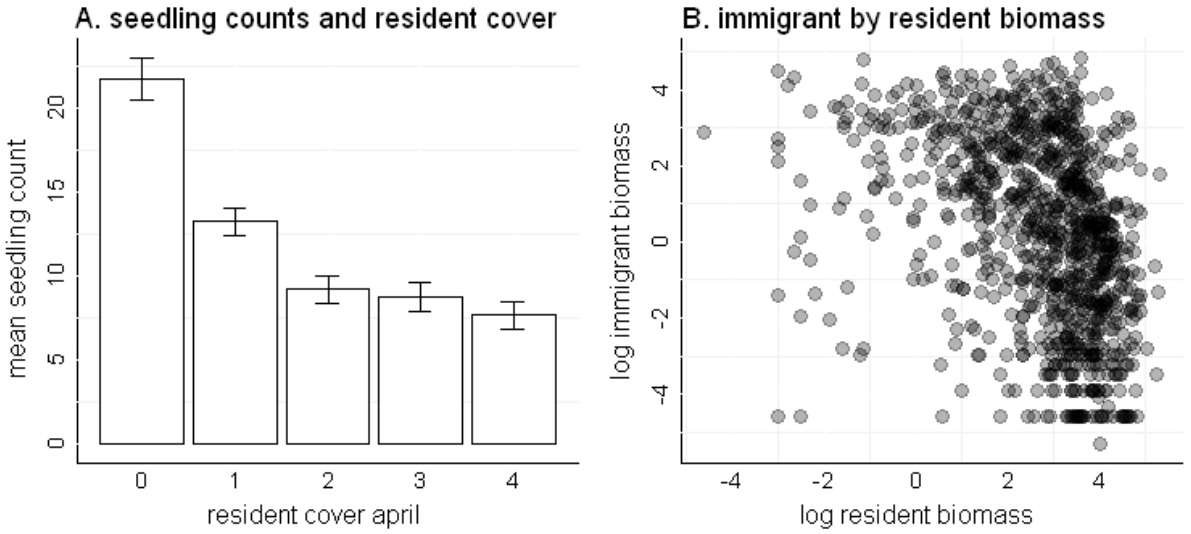

Figure 4. Mean immigrant seedling number (summed over two counts) against resident cover in April (A) and logged immigrant biomass (summed over two harvests) as a function of logged resident biomass (summed over two harvests) (B). Error bars in panel A = standard error of the mean.

Immigrant seedling numbers did not significantly differ between the two successional groups (Table 5 & 6). However, counts were different, on average there were almost double the number of individuals of early successional species (16.74±0.82 per 0.25 x 0.25 m) versus mid successional species (8.82±0.43 per 0.25 x 0.25 m), this difference was predominantly caused by a small number of early successional species being highly abundant. The seedling counts of different functional groups of immigrant species also responded differently according to their successional status, as indicated by a significant interaction between the successional group and the functional group of the immigrant (Table 5, $F_{2,1574} = 3.67$, $P < 0.0257$). This was essentially the result of early-successional grass species being in much greater abundance than mid-successional grass species, a trend weakly echoed by the legume species, and opposed by the forb species. The general response differences of immigrant functional groups to their successional status was also significant in the analyses of monocultures alone (Table 6, $F_{2,986} = 4.37$, $P = 0.0129$).

The successional status of the immigrant species was a more important factor explaining immigrant biomass. Immigrant biomass responded most to successional status, this being the most significant fixed effect in the analysis of both community types and the analysis of monocultures (Table 7, $F_{1,924} = 12.40$, $P = 0.0005$ & Table 8, $F_{1,719} = 11.22$, $P = 0.0009$). Mean immigrant biomass responded in a similar but more extreme pattern than the seedling counts. On average the collective weight of early successional species was 10.58±0.52 g per 0.25 x 0.25 m versus 1.73±0.20 g per 0.25 x 0.25 m for the mid successional species. Unlike the seedling counts, this trend was inconsistent across species pools, where two of the four early successional pools far outperformed the remaining six pools (pool was a random term in both models, Table 7 & 8). Immigrant biomass was also significantly different between the functional groups of immigrant species, for the analysis of both community types and the analysis of monocultures (Table 7, $F_{2,924} = 4.95$, $P = 0.0073$ & Table 8, $F_{2,719} = 5.13$, $P = 0.0061$). On average, established immigrant legume species produced the most immigrant biomass, 14.39±1.06 g per 0.25 x 0.25 m quadrat, versus 9.23±1.01 g per 0.25 x 0.25 m for the grass species, and 3.74±0.39 g per 0.25 x 0.25 m for the forb species.

Table 6. Variance components and a fixed effect ANOVA for a mixed effects model of mean seedling counts (averaged over two counts) for monocultures only. Statistical significance of fixed effects was determined using Markov chain Monte Carlo simulations to calculate probability estimates. Fixed effects that are significant at the 5 % threshold are shown in italics. Analysed with R (version 2.10.1).

Variance components for random terms	Variance	SD
block	0.0000	0.0000
pool	0.0101	0.1005
resident species	0.0574	0.2397
immigrant species	0.4854	0.6967
resident species x immigrant species	0.5909	0.7687
residual	0.3057	0.5529

Fixed effects	DF	SS	MS	F	DF2	P
cover april	*1*	*27.209*	*27.209*	*88.9951*	*986*	*<0.0001*
functional resident	2	1.302	0.651	2.1289	986	0.1195
succession	1	0.037	0.037	0.1213	986	0.7277
functional immigrant	2	0.723	0.362	1.1824	986	0.3070
nutrient	*3*	*4.260*	*1.420*	*4.6445*	*986*	*0.0031*
cover april x functional immigrant	*2*	*1.864*	*0.932*	*3.0484*	*986*	*0.0479*
functional resident x functional immigrant	*4*	*5.640*	*1.410*	*4.6118*	*986*	*0.0011*
succession x functional immigrant	*2*	*2.673*	*1.337*	*4.3714*	*986*	*0.0129*
cover april x nutrient	3	0.924	0.308	1.0078	986	0.3885
functional resident x nutrient	6	0.877	0.146	0.4782	986	0.8249
succession x nutrient	3	1.430	0.477	1.5591	986	0.1977
functional immigrant x nutrient	*6*	*8.373*	*1.396*	*4.5644*	*986*	*0.0001*
cover april x functional resident	*2*	*1.879*	*0.940*	*3.0736*	*986*	*0.0467*
cover april x succession	1	0.113	0.113	0.3700	986	0.5431
functional resident x succession	2	0.198	0.099	0.3233	986	0.7238
cover april x functional resident x succession	*2*	*1.987*	*0.994*	*3.2499*	*986*	*0.0392*
cover april x succession x nutrient	*3*	*2.412*	*0.804*	*2.6292*	*986*	*0.0490*

General effects of the resident-community on immigrant species

The resident community was measured in two ways, resident cover was recorded in April, May and July, and resident biomass was harvested twice. The cover measures for April generate a model with the lowest AIC explaining seedling counts (AIC = 4022.9), versus models using average resident biomass (AIC 4087.7) and the May (AIC = 4078.4) and July (AIC = 4110.8) cover measures. The biomass of the immigrants is best modelled by a summed measure of the resident biomass over the two harvests. Both with and without the diversity component (Tables 5 & 6) the April cover of resident species can be seen to have the greatest impact on the number of seedlings establishing. With increasing cover of resident species, there was a sustained decrease

in the average number of seedlings per 0.25 x 0.25 m quadrat from 21.73±1.28 in completely open plots (cover class = 0) to 7.64±0.80 in plots with full cover (cover class = 4) (Fig 4A). This effect was highly significant for the analysis of both community types (Table 5, $F_{1,1574} = 97.97$, $P<0.0001$) and the analysis of monocultures (Table 6, $F_{1,986} = 90.00$, $P < 0.0001$). In an analysis of both community types, resident biomass had the most significant effect on immigrant biomass (Table 7, $F_{1,924} = 16.01$, $P = 0.0001$). With increasing resident biomass there is a general decrease in immigrant biomass (Fig. 4B), this effect was weaker in the analysis of monocultures, but significant nonetheless (Table 8, $F_{1,719} = 6.09$, $P = 0.0138$).

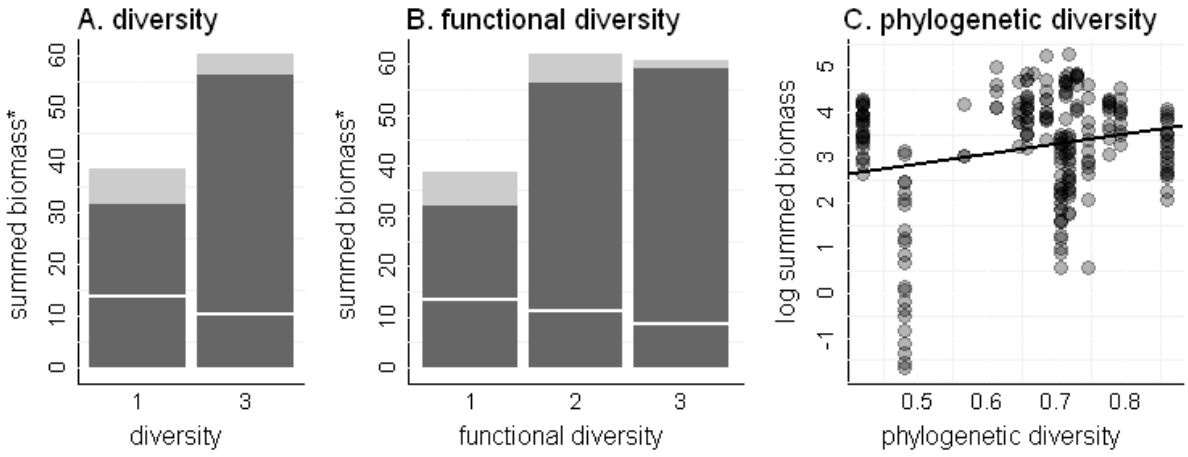

Figure 5. Mean biomass (averaged over two counts) against diversity (A) and functional diversity (B). Dark greys parts of each bar represent mean resident biomass, the light grey parts of each bar represent mean immigrant biomass, while the white horizontal line is the mean seedling number, which is on a count scale, with a range that falls within the biomass values (A & B). In panel C logged biomass of resident species is plotted against phylogenetic diversity, therefore monocultures are omitted.

Table 7. Variance components and a fixed effect ANOVA for a mixed effects model of immigrant biomass (summed over two harvests), for both monocultures and three-species communities. Statistical significance of fixed effects was determined using Markov chain Monte Carlo simulations to calculate probability estimates. The terms grass, forb and legume refer to the presence of each of these functional groups in the resident community (either in monoculture or mixture). The covariate resident biomass was logged to achieve normality of the residual scatter. Fixed effects that are significant at the 5 % threshold are shown in italics. Analysed with R (version 2.10.1).

Variance components for random terms	Variance	SD
block	0.0000	0.0000
pool	0.0000	0.0000
plot (resident species)	0.6325	0.7953
immigrant species	1.9267	1.3881
strip (resident species x immigrant species)	0.9349	0.9669
residual	1.1422	1.0687

Fixed effects	DF	SS	MS	F	DF2	P
resident biomass	*1*	*18.282*	*18.282*	*16.0056*	*924*	*0.0001*
diversity	*1*	*14.947*	*14.947*	*13.0862*	*924*	*0.0003*
succession	*1*	*14.168*	*14.168*	*12.4040*	*924*	*0.0005*
grass	1	2.891	2.891	2.5307	924	0.1120
forb	1	1.923	1.923	1.6838	924	0.1948
legume	1	1.580	1.580	1.3834	924	0.2398
functional immigrant	*2*	*11.309*	*5.655*	*4.9506*	*924*	*0.0073*
home/away	1	0.065	0.065	0.0567	924	0.8119
nutrient	3	3.401	1.134	0.9924	924	0.3956
resident mass x succession	*1*	*9.713*	*9.713*	*8.5034*	*924*	*0.0036*
forb x home/away	*1*	*7.267*	*7.267*	*6.3626*	*924*	*0.0118*
legume x home/away	*1*	*9.418*	*9.418*	*8.2458*	*924*	*0.0042*
resident mass x functional immigrant	*2*	*12.989*	*6.495*	*5.6861*	*924*	*0.0035*
diversity x functional immigrant	2	3.706	1.853	1.6221	924	0.1980
diversity x nutrient	3	5.725	1.908	1.6707	924	0.1717
functional immigrant x nutrient	*6*	*18.873*	*3.146*	*2.7539*	*924*	*0.0117*
home/away x nutrient	3	6.651	2.217	1.9409	924	0.1214
functional immigrant x home/away	*2*	*15.763*	*7.882*	*6.9003*	*924*	*0.0011*

The increase in resident diversity did not have a significant direct influence on immigrant seedling numbers (Fig. 5A & 14.04±0.67 per 0.25 x 0.25 m in monocultures versus 10.52±0.63 per 0.25 x 0.25 m in three-species communities). There was, however, a significant interaction between resident-community cover in April and diversity on seedling numbers, seedling numbers were more negatively affected by cover in the diverse plots than in the monoculture plots (Table 5, $F_{1,1574}$ = 11.21, P = 0.0008). As successional status applies to both resident and immigrant species in the same way, it is unsurprising that there are two three way interactions that include both a

measure of cover and succession (Table 5). A significant interaction occurred between resident-community cover in April, diversity and successional status (Table 5. $F_{1,1574}$ = 11.21, P = 0.0008), the negative effects of cover being strongest in three-species communities of early-(reduction from 23.42±3.08 g per 0.25 x 0.25 m quadrat under cover score 0 to 10.32±4.08 g per 0.25 x 0.25 m quadrat under cover score 4) and weakest in three-species communities of mid-successional communities, generally mid-successional communities produced a more stochastic trend, regardless of community diversity. Immigrant biomass was significantly lower in three-species communities (6.96±0.40 g per 0.25 x 0.25 m quadrat) than in monocultures (3.85±0.34 g per 0.25 x 0.25 m quadrat) (Fig. 5A & Table 7, $F_{1,924}$ = 13.09, P = 0.0003), however there was no significant interaction between diversity and resident-community biomass. Immigrant biomass was also significantly reduced with increasing functional diversity of three-species communities (Fig. 5B, $F_{1,923}$ = 7.23, P = 0.0007). Resident biomass has already been shown to increase with the increasing number of resident functional groups in a three-species community, however this decrease in immigrant biomass was not directly attributable to this as there was no significant interaction between resident functional group diversity and resident biomass. In a similar manner to the seedlings, successional status applies to both resident and immigrant species in the same way, and there is also a significant interaction between resident biomass and succession in explaining immigrant biomass. Mid-successional residents were in greater abundance or size (not distinguishable in a summed biomass measure) increasingly suppressing immigrants (Table 7 & 8). Both metrics of immigrant success, seedling counts ($F_{1, 232}$ = 7.43, P < 0.0069) and immigrant biomass ($F_{1, 188}$ = 4.56, P < 0.0360) revealed phylogenetic diversity to significantly reduce immigrant performance (Fig. A4 - for immigrant biomass).

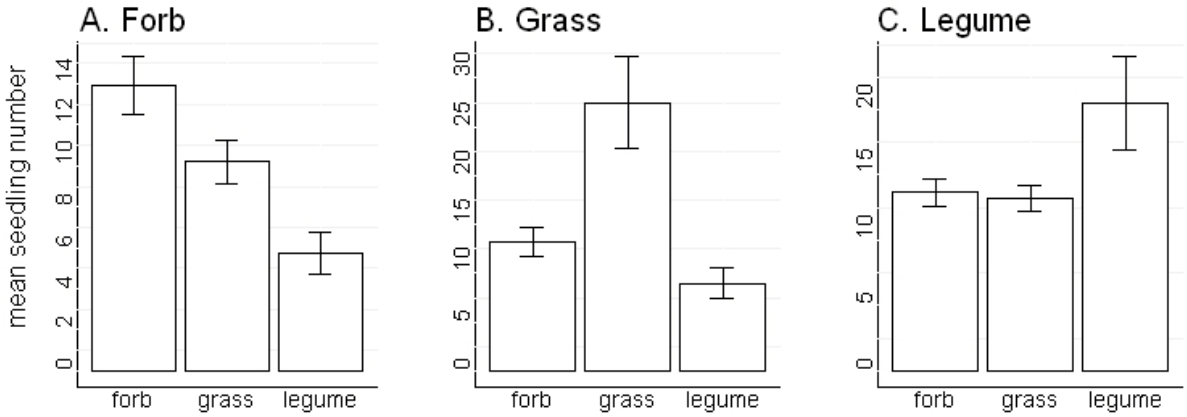

Figure 6. Mean immigrant seedling number (averaged over two counts) against the functional group of the resident, each panel represents a different functional group of immigrant species: forbs (A), grasses (B) and legumes (C). Error bars = standard error of the mean.

Table 8. Variance components and a fixed effect ANOVA for a mixed effects model of mean immigrant biomass (averaged over two harvests) for monocultures only. Statistical significance of fixed effects was determined using Markov chain Monte Carlo simulations to calculate probability estimates. The covariate resident biomass was logged to achieve normality of the residual scatter. Fixed effects that are significant at the 5 % threshold are shown in italics. Analysed with R (version 2.10.1).

Variance components for random terms	Variance	SD
block	0.0000	0.0000
pool	0.0000	0.0000
resident species	0.9249	0.9617
immigrant species	1.7913	1.3384
resident species x immigrant species	0.9883	0.9941
residual	1.1063	1.0518

Fixed effects	DF	SS	MS	F	DF2	P
resident biomass	*1*	*6.737*	*6.737*	*6.0902*	*719*	*0.0138*
functional resident	2	1.497	0.748	0.6765	719	0.5087
succession	*1*	*12.409*	*12.409*	*11.2173*	*719*	*0.0009*
functional immigrant	*2*	*11.351*	*5.675*	*5.1302*	*719*	*0.0061*
nutrient	3	5.284	1.761	1.5922	719	0.1899
resident biomass x functional immigrant	2	6.108	3.054	2.7607	719	0.0639
functional resident x functional immigrant	*4*	*21.554*	*5.389*	*4.8709*	*719*	*0.0007*
resident biomass x functional resident	*2*	*6.342*	*3.171*	*2.8663*	*719*	*0.0576*
resident biomass x succession	*1*	*8.448*	*8.448*	*7.6368*	*719*	*0.0059*

General effects of immigrant species on the resident-community

Increasing immigrant biomass significantly reduced resident cover in the combined analysis (Table 3, $F_{1,1592} = 11.58$, P=0.0007), as well as in the monoculture analysis, but less significantly so (Table 3, $F_{1,1069} = 5.69$, P=0.0172). A significant three way interaction was found between immigrant biomass, successional status, and immigrant functional group, in the analysis of monocultures alone (Table 3, $F_{1,1069} = 4.18$, P=0.0157). Immigrant biomass, and its interaction with the functional group of the immigrant, and the successional group of the immigrant, explained the majority of the remaining response of the resident-community biomass in both types of analyses (Table 3 & 4).

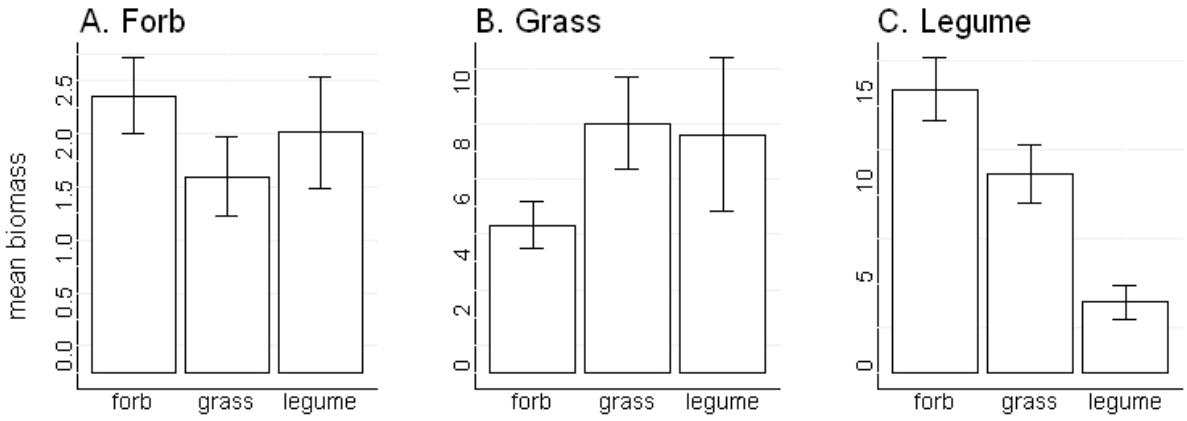

Figure 7. Mean immigrant biomass (averaged over two harvests) against the functional group of the resident, each panel represents a different functional group of immigrant species: forbs (A), grasses (B) and legumes (C). Error bars = standard error of the mean.

Biotic interactions between resident and immigrant species

The interaction between the functional group of the resident and the functional group of the immigrant targets biotic filtering. This interaction can be approached directly in the monoculture models, as the interaction between the resident and the immigrant functional groups. For both immigrant seedlings and biomass this interaction is significant (Table 6, $F_{4,986} = 4.61$, P = 0.0011 & Table 8, $F_{4,719} = 4.87$, P = 0.0007). Seedling counts of each functional immigrant on each of the three functional resident

species reveals that immigrants always perform best on their own functional group (Fig. 6). Almost three times the number of forb seedlings (13.89±1.41) establish in a community where the resident is a forb as opposed to in a legume community (5.75±1.04) (Fig. 6A). Similarly, three times the number of grass seedlings (27.44±4.21) establish in a community where the resident is a grass as opposed to in a legume community (8.98±1.58) (Fig. 6B). Legume seedlings also do better on plots where the resident species is a legume (20.48±3.54), a third better than if they would be establishing in forb (13.65±1.04) or grass communities (13.18±0.96) (Fig. 6C). In contrast to the seedlings, the biomass of immigrants suggests stochastic establishment responses in communities belonging to different functional groups, or for legume species, the opposite of the seedlings – increased biomass in communities belonging to different functional groups (Fig. 7). Forb biomass is highest in forb communities; however this is not significantly different to the performance of forbs in legume communities (Fig. 7A). Grass biomass is also highest in grass communities, but once more, this is not significantly different to the performance of grasses in legume communities (Fig. 7B). The biomass of legume immigrants contrasts patterns of seedling establishment, with the least amount of biomass being produced in legume communities (3.95±0.96), comparatively a third of what was produced in grass communities (11.13±1.64), and a quarter of that resulting from legume immigrants in forb communities (15.9± 1.74) (Fig. 7C). As this interaction is the key aspect of the experiment allowing insight into interspecific competitive processes, there is potential in looking at other aspects of the interaction. The interaction can be simplified, or split apart to the family level to accommodate the fact that the forb functional group is comprised of 8 families, while the grass and legume functional groups contain single families, or replaced with phylogenetic distance between resident and immigrant species. In simplifying the interaction, the interaction can be replaced with the binary factor home/away, coded for whether or not a functional group is entering a community where its own functional group is present (home) or absent (away). We can average across all species to produce a log ratio plot for both seedlings and biomass separated further by diversity (Fig. 8). The average seedling log ratios show that immigrants perform worse in home communities, and that this effect is enhanced with increasing

diversity, suggesting the responsible mechanism is intensified with diversity (a proxy for increasing competition) (Fig. 8A). In contrast, the average log ratios of immigrant biomass show immigrants to perform better in away communities, and this effect is again enhanced with increasing diversity (Fig. 8B). If we look at the metrics of immigrant success separately, for the seedling counts home/away is highly significant in models including both community types (Table 5, $F_{1,1574} = 33.09$, $P < 0.0001$) and for monocultures alone ($F_{1,978} = 18.73$, $P < 0.0001$), with the majority of species performing better in home communities. This relationship is easiest to visualise as a log ratio of immigrant performance in home communities by immigrant performance in away communities (Fig. 9A). In contrast to the seedling measures, immigrant biomass tends to show the opposite trend, with more than half of the species performing better in away communities (Fig. 9B), albeit non-significantly. To compensate for the forb functional group being made up of 8 families, while the grass and legume functional groups are comprised of just the Poaceae and Fabaceae respectively, we can use a factor of family home/away, coded for whether or not a species is entering a community where its own family is present (home) or absent (away). There is less freedom in doing this, as some of the forb families are represented by single individuals, and thus for the test of family home/away, the home test is the species itself. Nonetheless, for the seedling counts family home/away is a hugely significant effect ($F_{1,979} = 50.79$, $P < 0.0001$), with 35 of 46 species performing better in home communities (Fig. A6A). An even larger number of species perform better in family away communities for the biomass log ratio measures (Fig. A6B), but again non-significantly. A final alternative of this interaction is phylogenetic distance, a measure of the phylogenetic relatedness of the species pairs between monoculture resident and immigrant. This measure alone does not have a significant effect on seedling numbers, however there is a significant interaction between resident April cover and phylogenetic distance ($F_{1,807} = 8.29$, $P = 0.0041$), suggesting that as competition increased so did the importance of phylogenetic distance between species, however, this is actually caused by the difference in immigrant performance where cover is 0 versus the presence of a resident community. Phylogenetic distance did not explain immigrant biomass response, nor interact with any other factors.

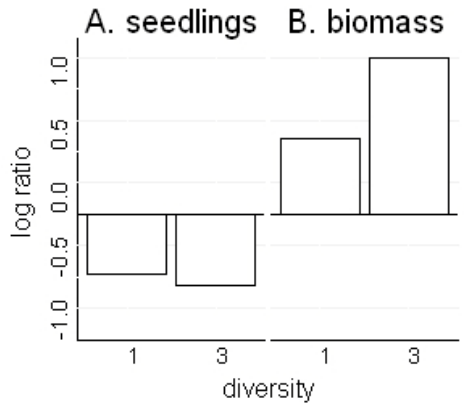

Figure 8. Log ratios based on performance of species in communities containing the same functional group as themselves (home) or not (away): immigrant seedling counts (A), immigrant biomass (B). A positive log ratio demonstrates a species performing better in an away community.

Variance components – species

Resident and immigrant species are present as random effects or variance components in all of these analyses, they are interesting to briefly mention as they allow the dominant driver of variance to be identified. We know that species are different and inherently variable. However, that the greater amount of variation can be explained by immigrant species for both models (Table 6 & 8) as opposed to resident species, suggests that immigration is driven by the immigrant, or its interaction with a resident species, but not solely the resident. For the seedling counts, it is the interaction between the immigrant and resident species that is even more important, explaining more of the variance than any of the other random terms (Table 6).

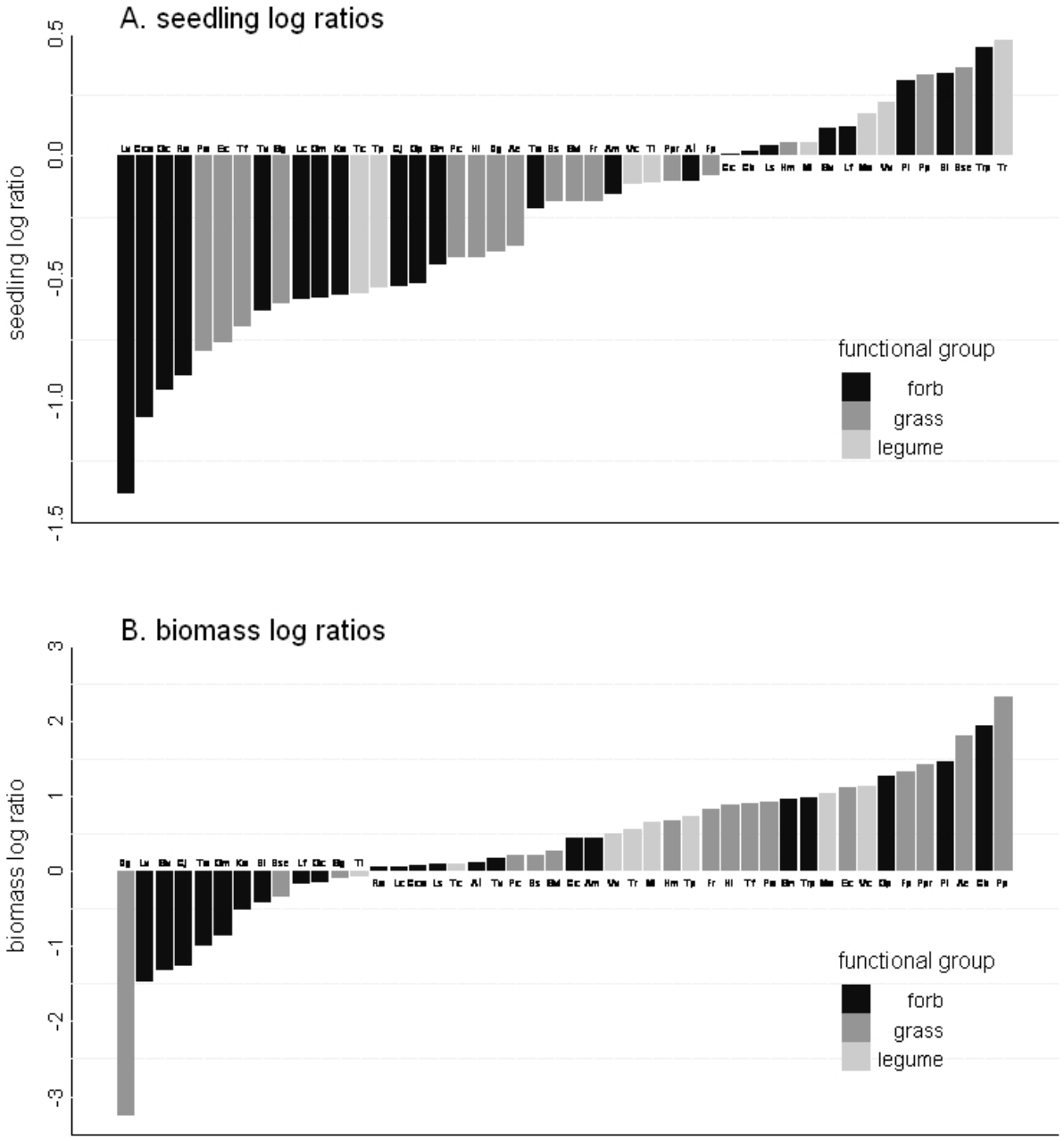

Figure 9. Log ratios based on performance of species in communities containing the same functional group as themselves (home) or not (away): immigrant seedling counts (A), immigrant biomass (B). A positive log ratio demonstrates a species performing better in an away community.

Discussion

Resident community

The size of the resident community was the major determinant of immigrant success for both immigrant metrics, with increasing resident community size reducing the number or size of immigrant species. This follows the expectation that the resident community will have an enormous effect on immigrants, as both generally compete for the same basic resources, light, water and nutrients, and resident species have the advantage of being much bigger with an established resource acquisition network. Both measures of immigrant success, seedling counts and immigrant biomass were influenced most by resident abundance (cover) and size (biomass and cover), respectively (Fig. 1). The interesting component of the influence of resident abundance and size is the interaction this covariate has with other factors.

Diversity

The increase in diversity from monocultures to three-species mixture significantly increased the summed biomass of the resident species (Fig. 2). Therefore a component of the diversity effect upon immigrant species was simply the biomass increase of the resident species. Diversity had no direct effect on immigrant seedling number, but did so indirectly through further increasing the size of the community. The latter metric of immigrant success – immigrant biomass – was significantly reduced by increasing diversity, independent of the increase in the size of the resident community. This reflects a change in the interaction between the immigrant and the resident community as the immigrant develops from seedling to established individual. Once the immigrant is physiologically mature it can be expected to compete for similar resources as members of the resident community, so the degree of interspecific competition will increase, and the more species there are in a community the more likely it is that one will share a similar resource niche to the immigrant. Many studies have shown increasing diversity to reduce invasion or immigration success (van Ruijven *et al.* 2003). Immigrants responded in the same way to functional diversity, which only broke down the gradient a little bit further, with diversity having no direct effect on immigrant seedling number, but did so indirectly through further increasing the size of the

community. And again, the latter metric of immigrant success – immigrant biomass – was significantly reduced by increasing functional diversity, again independent of the increase in the size of the resident community. The measure of phylogenetic diversity only included the three-species communities, as a community value of phylogenetic complexity can not be calculated for a single species (e.g. a monoculture). In both metrics of immigrant success, phylogenetic diversity significantly reduced immigrant biomass. This suggests that species diversity alone does not encapsulate all that is going on, and that at a finer evolutionary level, diversity has still a role to play. On the flip side resident biomass also significantly increased with increasing phylogenetic diversity but this increase in mass was not responsible for the decrease in immigrant performance.

Succession

The early successional species proved to be better immigrants than the mid successional species, most likely due to the ruderal qualities of their life history characteristics, fast growing, easily dispersed species, while the mid successional species had significantly bigger resident communities.

Nutrients

Resident communities also significantly increased with the addition of nitrogen, but not so with phosphorus. Seedling numbers were all reduced in plots with any form of nutrient addition, but in a complimentary measure to the resident increase, with smallest decrease being in the phosphorous treatment. Once established however, immigrant biomass increased with all nutrient additions, functional groups responded differently and accordingly with their physiological expectations, with grasses increasing most in nitrogen enriched plots, while legumes responded most to phosphorous addition. Nutrient responses suggest that increases in available nutrients do not necessarily favour immigrants, potentially having implications against theory supporting invasive species are facilitated by resource flushes, this is unlikely to be the case if an established community is present in a system. We need to be careful in lending these

results to questions relating to exotic invasive species, as the species used here will interact in accordance to having co-evolved.

Functional group interaction

The interaction between functional group of the immigrant and functional group of the resident is where the two metrics of immigrant performance most contrast one another. For both metrics, the interaction is significant, but the direction of the response varies. For the seedling counts, all three immigrant functional groups have the highest seedling numbers in communities where they are functionally resident, a positive feedback of belonging to that functional group. There are two basic mechanisms that could be responsible for this. A resident community may facilitate seedlings belonging to the same functional group, for example the conditions generated in a resident stand might afford a more optimal environment for seedlings belonging to that group. Alternatively, there is a belowground mutualistic biotic network that has established in association with the resident species over there three years of occupancy, and seedlings are plugging into this network. Such a mutualistic biotic network would have to be specialised enough to facilitate one functional group over another, they could be exhibiting phylogenetic host conservatism on the family level. There is some evidence suggesting the mutualistic species with plant hosts are more likely to be conserved at the family level. Of the mutualistic groups of species mycorrhizae would seem a likely candidate. Host selectivity of mycorrhiza has been shown to have various levels of specificity (Molina *et al*. 1992, Vandenkoornhuyse *et al*. 2003, Van Der Heijden & Horton 2009). In grasslands, for the few vascular species where mycorrhizal communities have been described, specialisation has been shown to be high, with strongly distinct communities found in association with vascular plant species coming from the same family (Vandenkoornhuyse *et al*. 2003). This might suggest that mycorrhiza are less likely to be facilitating vascular species belonging to the same functional group, however, there are still overlaps in the mycorrhiza species found to within vascular families, and the relative contribution of each mycorrhizal species to certain processes associated with their host plants is unknown. Other experiments have shown, that when various plant species collected from the field are grown in a pot to

establish a fungal network, and subsequently seedlings are introduced and their establishment monitored, then there is weak evidence that soil fungi (including AMF) of a specific host plant promoted it's own seedlings more than other seedling species (Van Der Heijden & Horton 2009). We are unable with this experiment to go beyond speculation as to the mechanism controlling this positive community feedback at the functional group level.

Somewhat in contrast to the seedling numbers, immigrant biomass produces a less certain story. Both forbs and grasses maintain the highest level of biomass in home communities, but they do not do so much better than in away communities, the different in performance varies little from the next best performance on a different functional group. The response of the legumes is quite different. Legume immigrant biomass measures indicate legumes having the least success in legume communities, home communities, but in away communities, where either forbs or grasses are the resident species, legumes perform much better, almost 3 times as well in grass communities and 4 times as well in forb communities. Various mechanisms might be responsible for such a feedback. Species are assigned to functional groups, as they share certain morphological and physiological traits, including similar means of resource uptake. Niche theory would then stipulate that a legume should not be as successful in a community of legumes, as the two have more similar resource requirements and will have an increased competitive interaction, where the growth of the non-established plant, the immigrant will suffer. Alternatively pests and pathogens may be phylogenetically conserved at the family level, and the accumulation of pests and pathogens after three years in the legume plots means an abundance of legume pests and pathogens will have adverse effects on establishing legume immigrants (sensu Petermann *et al.* 2008). However this would not explain the increase in the effect from monocultures to three-species communities (Fig. 5).

Another question is why it is only legumes that exhibit this strong negative feedback phenomenon? In other experiments in grasslands such negative feedbacks have been identified for all functional groups (Petermann *et al.* 2008, Petermann *et al.* 2010). Therefore it is more likely a component of this experiment than an aspect of the legume functional group that is limiting this effect to being a legume effect. The most

likely reason is the degree of competition intensity. In this experiment the diversity gradient was very low, plots either contained 1 or 3 species, compared to other more realistic experiments where species numbers are higher the intensity of competition is in turn greater. In other such experiments the intensity of the negative feedback has also been shown to coincide with the size of the species, the greater biomass of the species the greater the negative feedback (Petermann *et al.* 2010). Legumes are the most abundant functional group in this experiment, they produce more biomass both as residents and immigrants, this increase in competition intensity could be responsible for the negative feedback observed. Both mechanisms of competition for resources and the build up of negative soil pathogens and pests are density dependent, suggesting that the densities reached by the grass and forb groups in this experiment are unlikely to have been high enough for such mechanisms to come into operation.

If we consider legumes once more in terms of nutrients, it is the association with rhizobia permitting fixation of atmospheric nitrogen that is the most significant difference between legumes and other functional groups. Legumes should have abundant access to nitrogen, therefore they should compete more for other limiting nutrients. If we look at the legume group alone and its home away response crossed with the nutrient addition (Fig. A7) it is evident that in phosphorous plots, the main limiting resource for legumes, the home away effect is at its greatest. This suggests that resident species will usurp a limiting resource and in doing so limit it to species trying to establish that have a similar resource requirement. This has been shown already in nutrient poor environments, but not in fertile conditions such as this experiment was conducted under (Fargione *et al.* 2003).

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Appendix A.

Table A1: The species and functional group composition of the eight non-overlapping pools and the sixteen non-overlapping 3-species mixtures.

Pool	Small pool	Species	Functional group	Family
1	1.1	*Bromus sterilis*	grass	Poaceae
1	1.1	*Vicia villosa*	legume	Fabaceae
1	1.1	*Galinsoga ciliata*	forb	Asteraceae
1	1.2	*Echinochloa crus-galli*	grass	Poaceae
1	1.2	*Diplotaxis tenuifolia*	forb	Brassicaceae
1	1.2	*Lepidium campestre*	forb	Brassicaceae
2	2.1	*Setaria glauca*	grass	Poaceae
2	2.1	*Berteroa incana*	forb	Brassicaceae
2	2.1	*Conyza canadiensis*	forb	Asteraceae
2	2.2	*Poa annua*	grass	Poaceae
2	2.2	*Melilotus alba*	legume	Fabaceae
2	2.2	*Lactuca serriola*	forb	Asteraceae
3	3.1	*Hordeum murinum*	grass	Poaceae
3	3.1	*Trifolium incarnatum*	legume	Fabaceae
3	3.1	*Lepidium virginicum*	forb	Brassicaceae
3	3.2	*Panicum capillare*	grass	Poaceae
3	3.2	*Rumex acetosella*	forb	Polygonaceae
3	3.2	*Tanacetum vulgare*	forb	Asteraceae
4	4.1	*Setaria viridis*	grass	Poaceae
4	4.1	*Bromus secalinus*	grass	Poaceae
4	4.1	*Arctium tomentosum*	forb	Asteraceae
4	4.2	*Trifolium campestre*	legume	Fabaceae
4	4.2	*Senecio vernalis*	forb	Asteraceae
4	4.2	*Centaurea cyanus*	forb	Asteraceae
5	5.1	*Arrhenaterum elatius*	grass	Poaceae
5	5.1	*Festuca rubra*	grass	Poaceae
5	5.1	*Trifolium pratense*	legume	Fabaceae
5	5.2	*Galium mollugo*	forb	Rubiaceae
5	5.2	*Leucanthemum vulgare*	forb	Asteraceae
5	5.2	*Taraxacum officinale*	forb	Asteraceae
6	6.1	*Poa pratensis*	grass	Poaceae
6	6.1	*Medicago lupulina*	legume	Fabaceae
6	6.1	*Centaurea jacea*	forb	Asteraceae
6	6.2	*Phleum pratense*	grass	Poaceae
6	6.2	*Knautia arvensis*	forb	Dipsaceae
6	6.2	*Plantago lanceolata*	forb	Plantaginaceae
7	7.1	*Trisetum flavescens*	grass	Poaceae
7	7.1	*Trifolium repens*	legume	Fabaceae
7	7.1	*Lychnis flos-cuculi*	forb	Caryophyllaceae
7	7.2	*Holcus lanatus*	grass	Poaceae
7	7.2	*Silene nutans*	forb	Caryophyllaceae
7	7.2	*Tragopogon pratensis*	forb	Asteraceae
8	8.1	*Dactylis glomerata*	grass	Poaceae
8	8.1	*Vicia cracca*	legume	Fabaceae
8	8.1	*Crepis biennis*	forb	Asteraceae

8	8.2	*Festuca pratensis*	grass	Poaceae
8	8.2	*Achillea millefolium*	forb	Asteraceae
8	8.2	*Geranium pratense*	forb	Geraniaceae

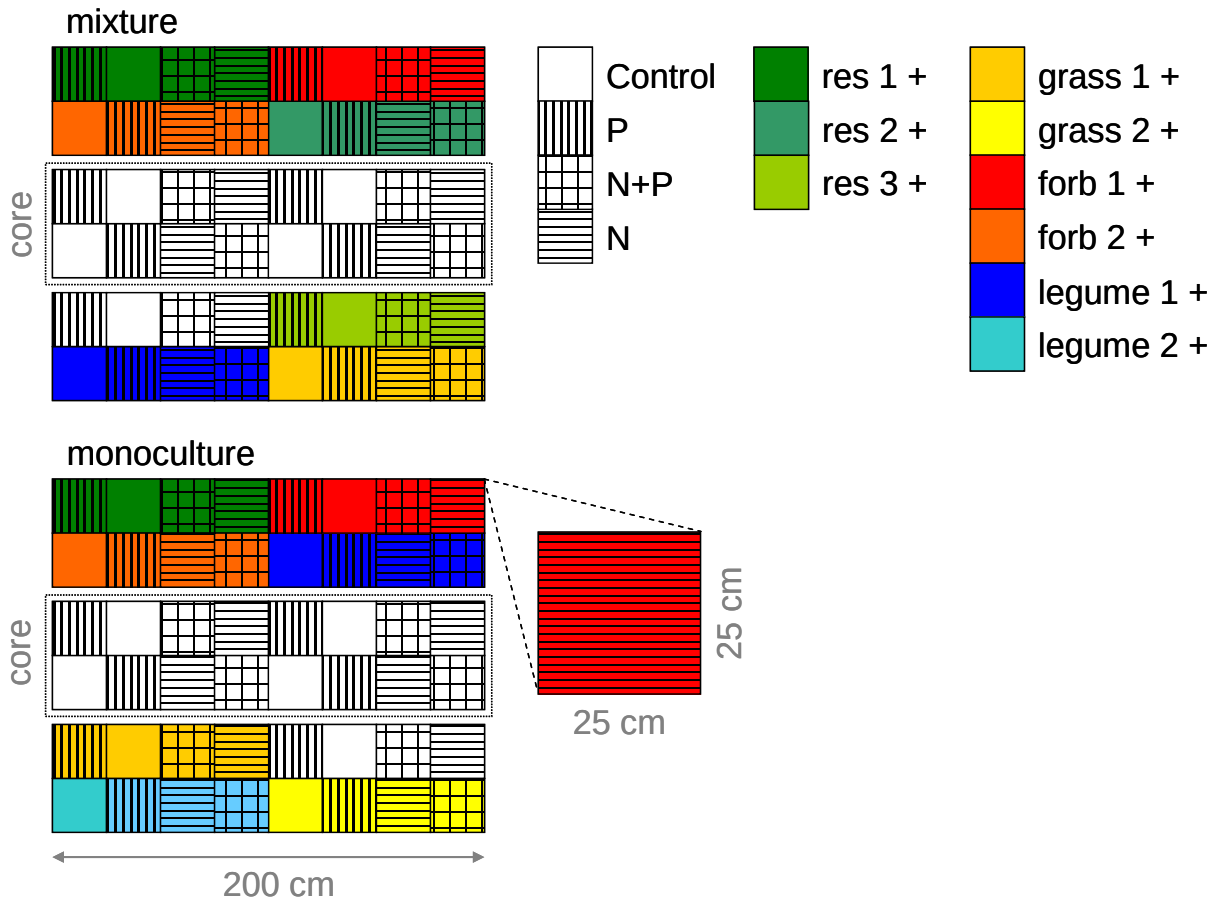

Figure A1. Spatial arrangement of the nutrient patches, and seed addition strips. Squares without pattern represent control patches (without fertiliser), horizontally striped squares represent phosphorous patches, vertically striped squares represent nitrogen patches and hatched squares represent patches with both nitrogen and phosphorous added. The light green, pale green, and dark green strips represent the addition of each of the resident species; note the single addition in the monocultures. Red and orange strips denote the addition of the first and second additional forb species. Dark blue and light blue strips represent the addition of the first and second additional legume species, and yellow and cream strips represent the addition of first and second additional grass species. The centre strips in white indicate the untouched core area.

			G	G	F	F	F	L	G	G	F	F	F	L	G	G	F	F	F	L	G	G	F	F	F	L
			1	1	1	1	1	1	2	2	2	2	2	2	3	3	3	3	3	3	4	4	4	4	4	4
			Bs	Ec	Dt	Gc	Lc	Vv	Pa	Sg	Bi	Cc	Ls	Ma	Hm	Pc	Lv	Ra	Tv	Ti	Bse	Sv	At	Ccy	Sv	Tc
G	1	Bs	X	X	X	X	X	X						X												
G	1	Ec	X	X	X	X	X	X						X												
F	1	Dt	X	X	X	X	X	X						X												
F	1	Gc	X	X	X	X	X	X						X												
F	1	Lc	X	X	X	X	X	X						X												
L	1	Vv	X	X	X	X	X	X						X												
G	2	Pa						X	X	X	X	X	X	X												
G	2	Sg						X	X	X	X	X	X	X												
F	2	Bi						X	X	X	X	X	X	X												
F	2	Cc						X	X	X	X	X	X	X												
F	2	Ls						X	X	X	X	X	X	X												
L	2	Ma						X	X	X	X	X	X	X												
G	3	Hm													X	X	X	X	X	X						X
G	3	Pc													X	X	X	X	X	X						X
F	3	Lv													X	X	X	X	X	X						X
F	3	Ra													X	X	X	X	X	X						X
F	3	Tv													X	X	X	X	X	X						X
L	3	Ti													X	X	X	X	X	X						X
G	4	Bse																		X	X	X	X	X	X	X
G	4	Sv																		X	X	X	X	X	X	X
F	4	At																		X	X	X	X	X	X	X
F	4	Ccy																		X	X	X	X	X	X	X
F	4	Sv																		X	X	X	X	X	X	X
L	4	Tc																		X	X	X	X	X	X	X
			G	G	F	F	F	L	G	G	F	F	F	L	G	G	F	F	F	L	G	G	F	F	F	L
			5	5	5	5	5	5	6	6	6	6	6	6	7	7	7	7	7	7	8	8	8	8	8	8
			Ae	Fr	Gm	Lv	To	Tp	Pp	Ppr	Cj	Ka	Pl	Ml	Hl	Tf	Lf	Sn	Tp	Tr	Dg	Fp	Am	Cb	Gp	Vc
G	5	Ae	X	X	X	X	X	X						X												
G	5	Fr	X	X	X	X	X	X						X												
F	5	Gm	X	X	X	X	X	X						X												
F	5	Lv	X	X	X	X	X	X						X												
F	5	To	X	X	X	X	X	X						X												
L	5	Tp	X	X	X	X	X	X						X												
G	6	Pp						X	X	X	X	X	X	X												
G	6	Ppr						X	X	X	X	X	X	X												
F	6	Cj						X	X	X	X	X	X	X												
F	6	Ka						X	X	X	X	X	X	X												
F	6	Pl						X	X	X	X	X	X	X												
L	6	Ml						X	X	X	X	X	X	X												
G	7	Hl													X	X	X	X	X	X						X
G	7	Tf													X	X	X	X	X	X						X
F	7	Lf													X	X	X	X	X	X						X
F	7	Sn													X	X	X	X	X	X						X
F	7	Tp													X	X	X	X	X	X						X
L	7	Tr													X	X	X	X	X	X						X
G	8	Dg																		X	X	X	X	X	X	X
G	8	Fp																		X	X	X	X	X	X	X
F	8	Am																		X	X	X	X	X	X	X
F	8	Cb																		X	X	X	X	X	X	X
F	8	Gp																		X	X	X	X	X	X	X
L	8	Vc																		X	X	X	X	X	X	X

Figure A2. Diallel of interspecific interactions.

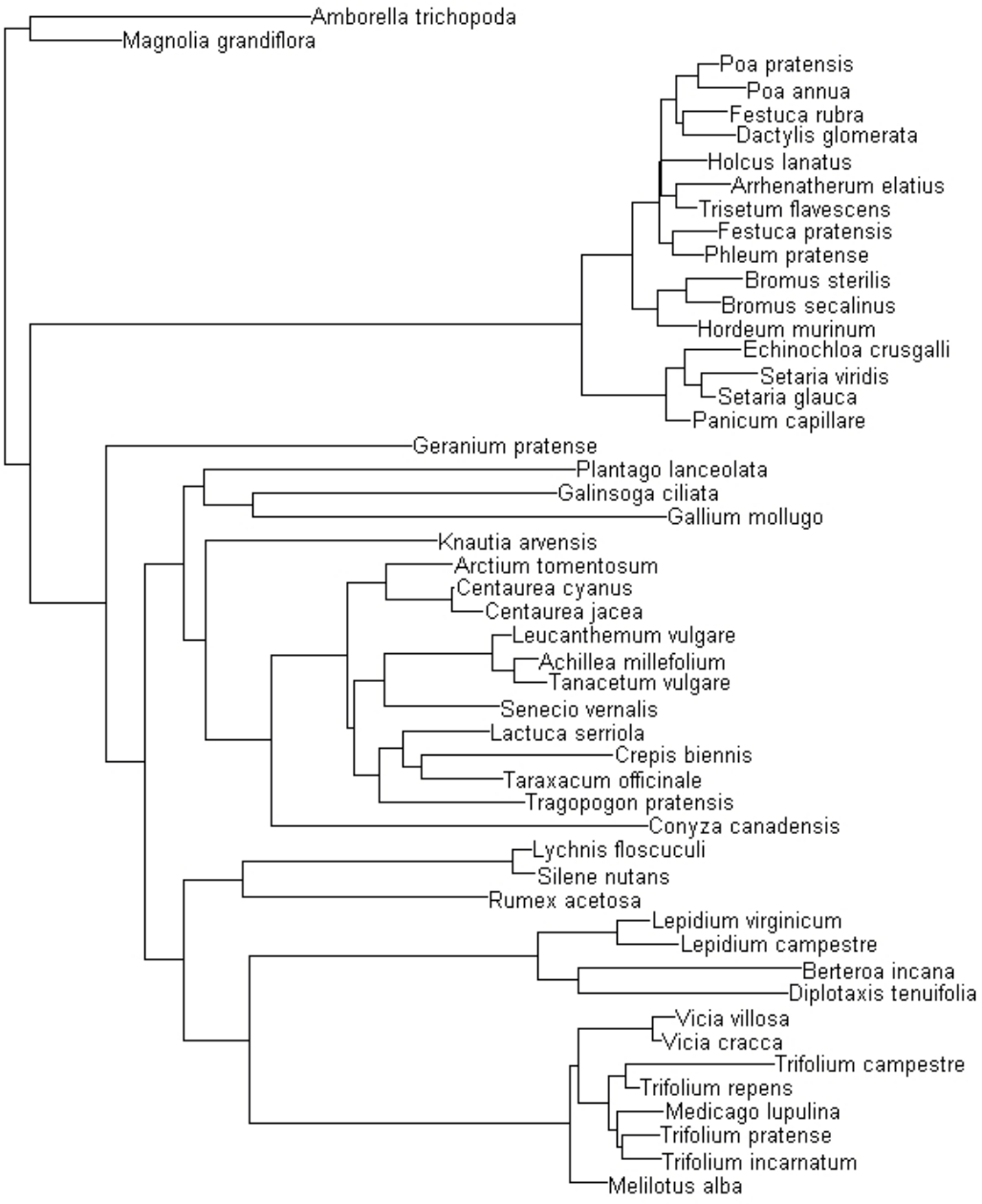

Figure A3. Phylogeny of the 48 experimental species with *Amborella trichopoda* and *Magnolia grandiflora* as outgroups.

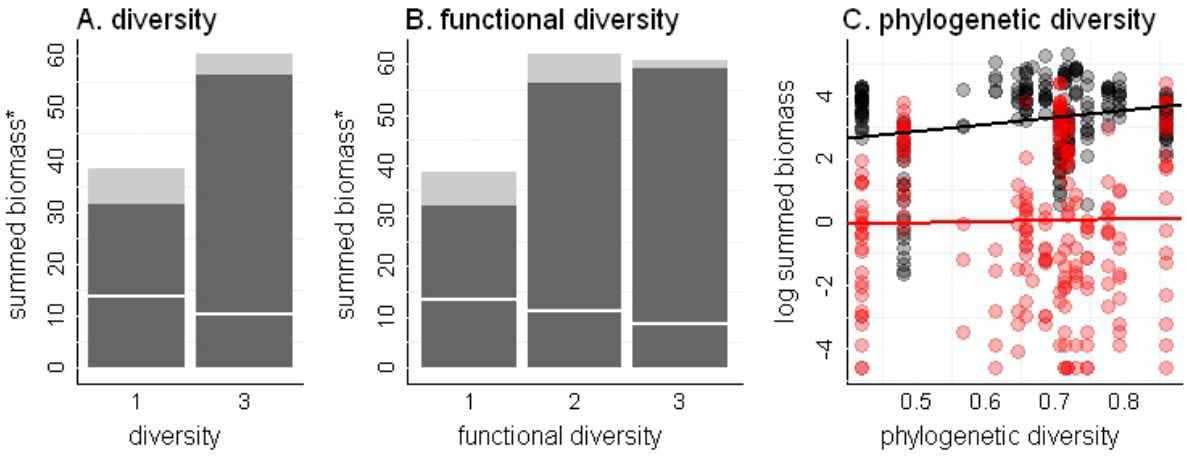

Figure A4. (version of figure 2) Mean biomass (averaged over two counts) against diversity (A) and functional diversity (B). Dark greys parts of each bar represent mean resident biomass, the light grey parts of each bar represent mean immigrant biomass, while the white horizontal line is the mean seedling number*, which is on a count scale, with a range that falls within the biomass values (A & B). In panel C logged biomass of resident (black points) and immigrant species (red points) is plotted against phylogenetic diversity, therefore monocultures are omitted.

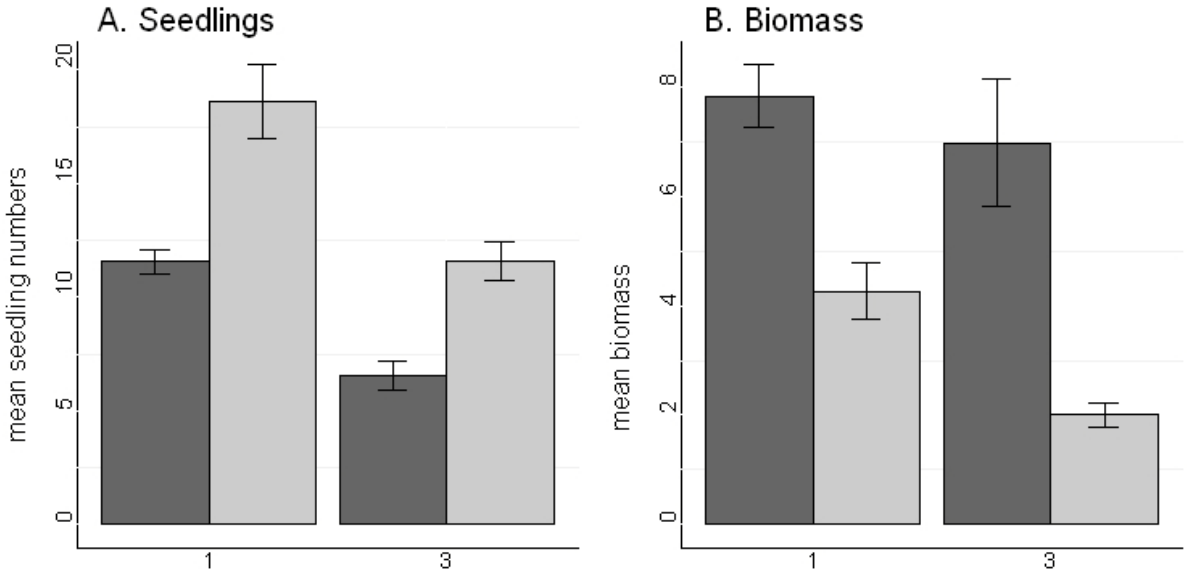

Figure A5. (version of figure 2) Mean biomass (averaged over two counts) against diversity (A) and functional diversity (B). Dark greys parts of each bar represent mean resident biomass, the light grey parts of each bar represent mean immigrant biomass, while the white horizontal line is the mean seedling number*, which is on a count scale, with a range that falls within the biomass values (A & B).

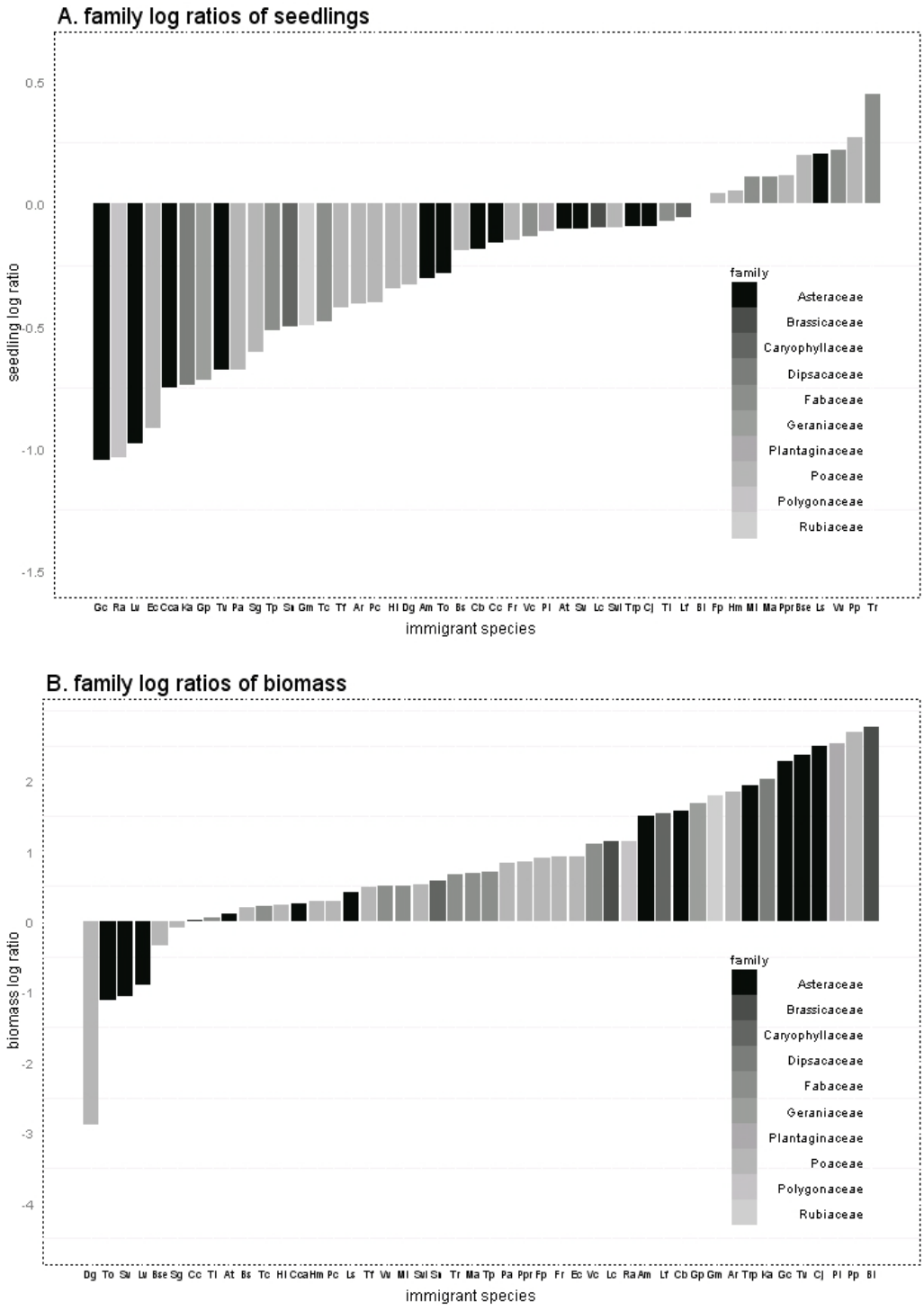

Figure A6. Log ratios based on performance of species in communities containing the same family as themselves (home) or not (away): immigrant seedling counts (A), immigrant biomass (B). A positive log ratio demonstrates a species performing better in an away community.

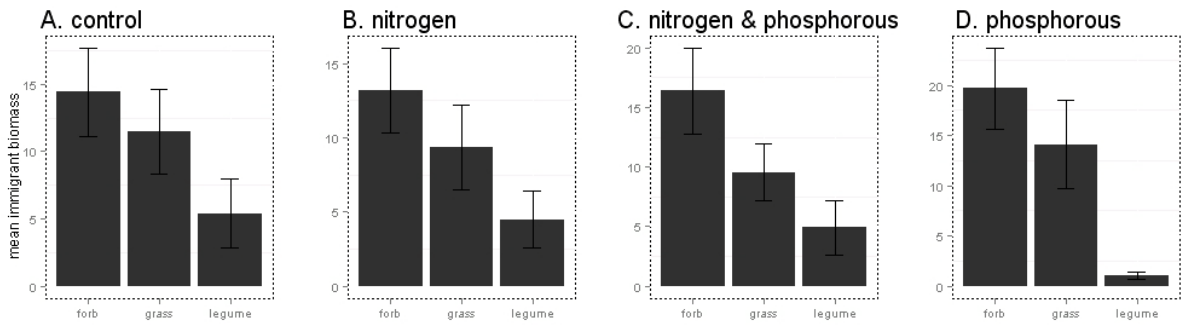

Figure A7. Mean immigrant biomass (averaged over two harvests) of legumes only against the functional group of the resident, each panel represents a different nutrient treatment: control (A), nitrogen (B), nitrogen & phosphorous (C), and phosphorous (D). Nutrient addition quantity: nitrogen = 2 x 8 g/m² (annually); phosphorous = 2 x 4 g/m² (annually); nitrogen & phosphorous = 2 x 4 g/m² N and 2 g/m² P (annually). Error bars = standard error of the mean.

Chapter 6

Species diversity reduces invasion success in pathogen-regulated communities.

Turnbull, L.A., Levine, J., Fergus, A.J.F. & Petermann, J.S. 2010. *Oikos* **119**: 1040-1046.

Abstract

The loss of natural enemies is thought to explain why certain invasive species are so spectacularly successful in their introduced range. However, if losing natural enemies leads to unregulated population growth, this implies that native species are themselves normally subject to natural enemy regulation. One possible widespread mechanism of natural enemy regulation is negative soil feedbacks, in which resident species growing on home soils are disadvantaged because of a build-up of species-specific soil pathogens. Here we construct simple models in which pathogens cause resident species to suffer reduced competitive ability on home soils and consider the consequences of such pathogen regulation for potential invading species. We show that the probability of successful invasion and its timescale depend strongly on the competitive ability of the invader on resident soils, but are unaffected by whether or not the invader also suffers reduced competitive ability on home soils (i.e. pathogen regulation). This is because, at the start of an invasion, the invader is rare and hence mostly encounters resident soils. However, the lack of pathogen regulation does allow the invader to achieve an unusually high population density. We also show that increasing resident species diversity in a pathogen-regulated community increases invasion resistance by reducing the frequency of home-site encounters. Diverse communities are more resistant to invasion than monocultures of the component species: they preclude a greater range of potential invaders, slow the timescale of invasion and reduce invader population size. Thus, widespread pathogen regulation of resident species is a potential explanation for the empirical observation that diverse communities are more invasion resistant.

Introduction

The loss of pathogens, herbivores and predators is commonly believed to underlie the success of some exotic plant species in their introduced range (the enemy release hypothesis: Elton 1958, Keane and Crawley 2002, Mitchell and Power 2003, Torchin and Mitchell 2004, Theoharides and Dukes 2007). However, if the loss of natural enemies is presumed to cause unregulated population growth, this implies that native species normally experience natural enemy regulation. This contrasts with the prevailing

view of many plant community ecologists who have traditionally emphasised resource-based mechanisms of coexistence (Tilman 1982, Grime 2001, Tilman et al. 2001, Cardinale et al. 2007).

Recent empirical work has shown that native plant species often suffer from negative soil feedbacks, a type of density-dependent regulation imposed by species-specific soil herbivores and pathogens (van der Putten et al. 1993, Bever 1994, Klironomos 2002, De Deyn et al. 2003, Bartelt-Ryser et al. 2005, Kardol et al. 2006). After a given plant species occupies a site for some time, specialist soil pathogens accumulate and reduce the performance of conspecific plants in subsequent generations – a type of Janzen-Connell effect (Janzen 1970, Connell 1971). The strength of these feedbacks is usually measured by comparing the performance of individuals on soils formerly occupied by the same species (home sites) or on soils formerly occupied by other species (away sites). Negative feedbacks have been reported from a variety of communities and vary considerably in strength (Kulmatiski et al. 2008). For example, in field-trained soils, Petermann et al. (2008) found that species from three different functional groups only achieved half the biomass on home soils versus away soils when grown in competition with other functional groups, although others have found weaker effects (Engelkes et al. 2008). Thus, pathogen in regulation – the form of negative soil feedbacks – is sufficiently widespread to warrant serious consideration as an alternative to resource-based mechanisms of coexistence (Kulmatiski et al. 2008, Petermann et al. 2008).

Invasive plants in their introduced range have often been found to suffer weaker negative soil feedbacks than their native competitors, suggesting that a lack of regulation by soil pathogens could be critical to their success (Klironomos 2002, Callaway et al. 2004, but see Beckstead and Parker 2003, Eppinga et al. 2006). For example, some have argued that freedom from negative feedbacks aids expansion of species into new territory (van Grunsven et al. 2007, Engelkes et al. 2008, Menendez 2008, but see Levine et al. 2006, Eppstein and Molofsky 2007). However, because invaders must begin from low population density where home-site encounters are rare, we hypothesise that freedom from negative feedbacks is unlikely to increase the

probability of successful invasion. We instead believe that the key ingredient to successfully invading resident communities is good competitive ability on resident soils.

We also hypothesise that soil pathogen regulation might interact with resident species diversity to influence invasion success. If negative feedbacks act on resident species then monocultures are likely to be particularly susceptible to invasion: in monocultures, resident species only encounter home sites where their performance is weakest. In contrast, in diverse communities, each resident species provides away sites on which the remaining residents can compete strongly; hence resident species in diverse communities largely avoid negative soil feedbacks. We might therefore expect that an invader would find it more difficult to invade diverse pathogen-regulated communities. This is consistent with a large body of empirical evidence showing that more diverse communities are indeed more difficult to invade (Knops et al. 1999, Naeem et al. 2000, Hector et al. 2001, Fargione et al. 2003, van Ruijven et al. 2003, Levine et al. 2004). While such results are usually attributed to more complete resource use in diverse communities, the role of soil feedbacks in explaining these patterns has not been explored.

Here we use simple models to explore the requirements for successful invasion when the resident plant community is regulated by negative soil feedbacks. We focus on simulation models because analytical solutions for multi-species systems are difficult, although we do provide analytical support for our invasion conditions (for a detailed theoretical treatment of the two-species case see Eppstein and Molofsky 2007). We first examine the impact of changing the strength of the negative feedback experienced by the invader on its probability of invasion, its rate of population increase and its equilibrium abundance. Second, we consider the effect of changing resident diversity on these same three measures of invasion success.

Methods and results

We consider invasion into a community of 100 000 sites each occupied by a single adult plant, whose dynamics are governed by a weighted lottery (Chesson and Warner 1981). Each year all plants in the community produce the same number of seeds, suffer the same probability of mortality (d_i= 0.2) and compete for the sites vacated by the death of

adults. The proportion of newly-vacated sites won by a given species is proportional to the product of its relative abundance in the community and its competitive ability, α. The value of α varies between the invader (denoted by an i subscript) and the resident (denoted by an r subscript) and between home and away soils, generating four values: $\alpha_{i,\ home}$, $\alpha_{i,\ away}$, $\alpha_{r,\ home}$, $\alpha_{r,\ away}$. Sites thereby carry a memory of the former occupant which influences future competitive interactions on that site. Based on the findings of Petermann et al. (2008), we assume that pathogen-driven negative soil feedbacks reduce competitive ability by half on home soils. Thus, for all simulations, we arbitrarily set the competitive ability of the resident species on away soils, $\alpha_{r,\ away}$ to be 0.4, and the competitive ability of the resident species on its home soil to exactly half this value, $\alpha_{r,\ home} = 0.2$ To examine the influence of invader competitive ability on its success, we varied invader performance on away soils, $\alpha_{i,\ away}$ over the interval 0.15–0.85 in steps of 0.05. To examine the effects of negative feedbacks on invader success, the invader either experienced no negative soil feedback: $\alpha_{i,\ home}=\alpha_{i,\ away}$ or, the invader suffered the same magnitude of negative soil feedback as the residents: $\alpha_{i,\ home}= 0.5\ \alpha_{i,\ away.}$

For simplicity, dispersal is global, meaning that the chance of a given species winning a site is a function of its proportion in the community at large, not its local proportion. Strongly limited dispersal would undoubtedly affect model outcomes (Eppstein and Molofsky 2007); however, we previously found that results were unaffected by the inclusion of local dispersal as long as > 50% of the seeds produced by each parent disperse away from the parent site (Petermann et al. 2008). All invasions were initiated with 16 invader individuals (~ 0.02% of the community). For each implementation of the model we recorded 1) whether or not the invasion succeeds; 2) the number of generations required for the invader to reach 1000 individuals (1% of the total community) and 3) the final population size of both the resident and the invader. An invasion is judged successful if at least one individual remains 2000 generations after introduction. For any given set of parameters, we performed 1000 repeated runs from identical starting conditions.

Invasion into a system with a single resident species

In a monoculture, all sites initially consist of home sites for the resident, while a rare invader initially encounters only away sites. As a consequence, whether or not the invader itself possesses a negative feedback has a negligible effect on its probability of successful invasion or the time required to reach 1000 sites (Fig. 1a–b). Of much greater importance is the invader's general competitive ability ($\alpha_{i,\ away}$), which strongly increases its probability of success (Fig. 1a) and decreases the time required to reach 1000 individuals (Fig. 1b).

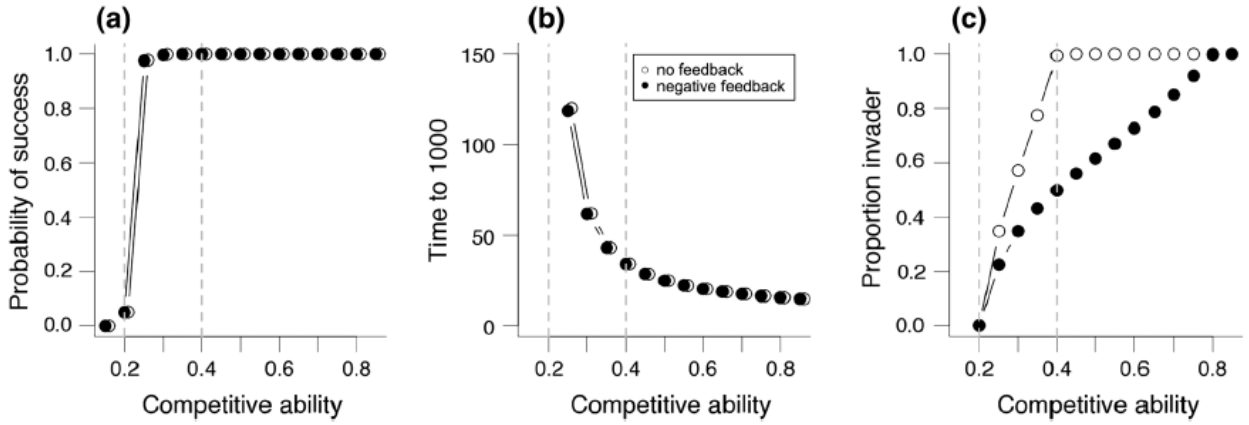

Figure 1. Success of an invader with and without a negative soil feedback and with different competitive abilities on 'away' soils. The probability that the invasion succeeds (a) the time-scale of successful invasion (b) and the proportion invader should the invasion succeed (c) are shown. Dashed lines show the competitive ability of the single resident species on home (0.2) and away soils (0.40). All values are calculated from 1000 repeated runs. The invader population size was calculated 2000 generations after introduction using data from successful invasions only (success constitutes >1 individual after 2000 generations). The timescale is the number of generations required for the invader to reach 1000 individuals.

However, should the invader satisfy the condition for successful invasion, the equilibrium population size of the invader is much larger when the invader lacks its own negative feedback (Fig. 1c). The equilibrium abundance of the invader is determined by the relative competitive abilities of the invader and the resident on home and away soils (Fig. 1c). For $0.2 < \alpha_{i,\,away} < 0.4$ the two species coexist, even when the invader lacks soil pathogen regulation; although without soil pathogen regulation the invader is more abundant than the resident. Similarly, an invader without soil pathogen regulation can exclude the resident when $\alpha_{i,\,away} > 0.4$ because it can outcompete the resident on both home and away sites (the resident's competitive ability never exceeds 0.4). However, a pathogen-regulated invader requires a higher minimum competitive ability on resident soils to exclude the resident, $\alpha_{i,\,away} > 0.8$. All of these thresholds can be analytically derived for this model, as shown below.

Analytical conditions for invasion and impact

The simulation results are supported by analytical equations describing the same dynamics but over an infinitely large number of sites. The proportion of sites occupied by the invader, p_i, changes from one time step to the next as follows:

$$p_{i,\,t+1} = (1-d)p_{i,t} + d\left[p_{i,t}\frac{\alpha_{i,home}p_{i,t}}{\alpha_{i,home}p_{i,t} + (1-p_{i,t})\alpha_{r,away}} + (1-p_{i,t})\frac{\alpha_{i,away}p_{i,t}}{\alpha_{i,away}p_{i,t} + (1-p_{i,t})\alpha_{r,\,home}}\right] \quad (1)$$

The first term of the sum describes the proportion of invader individuals surviving over the time step, while the second term is the proportion of newly-vacated sites subsequently filled by the invader. The proportion of newly-vacated sites filled by the invader is a weighted average of dynamics on sites that were formerly occupied by the invader (the first term of the bracketed sum) and dynamics on sites formerly occupied by the resident (the second term of the bracketed sum). The invader wins sites in

proportion to the product of its abundance and competitive ability, relative to the product of these values for the resident.

To obtain the condition for the invader to increase from rarity, we divide both sides of Eq. 1 by $p_{i,t}$, yielding the per capita growth rate for the invader:

$$\frac{p_{i,t+1}}{p_{i,t}} = (1-d) + d\left[p_{i,t} \frac{\alpha_{i,home}}{\alpha_{i,home} p_{i,t} + (1-p_{i,t})\alpha_{r,away}} + (1-p_{i,t}) \frac{\alpha_{i,away}}{\alpha_{i,away} p_{i,t} + (1-p_{i,t})\alpha_{r,home}} \right] \quad (2)$$

When the invader is rare, $p_{i,t}$ is near zero, simplifying the growth rate to:

$$\frac{p_{i,t+1}}{p_{i,t}} = (1-d) + d\left[\frac{\alpha_{i,away}}{\alpha_{r,home}} \right] \quad (3)$$

For the invader to increase when rare, this growth rate must exceed one, and simplifying yields the invasion condition:

$$\alpha_{i,away} > \alpha_{r,home} \quad (4)$$

Thus, the invader can successfully increase when rare if it can outcompete the resident on the resident's home soil. Importantly, condition 4 does not contain $\alpha_{i,\,home}$ and hence a negative soil feedback for the invader will not affect its probability of successful invasion or its dynamics when rare (Fig. 1a–1b). Condition 4 also explains why the threshold for successful invasion in the simulations is $\alpha_{i,\,away} > 0.2$ (as $\alpha_{r,\,home} = 0.2$ in the simulations).

Equations 1–4 above can also be used to describe the dynamics of the resident species by switching the i and r subscripts. We can thus derive the condition for the resident to persist with the invader (as the resident must also be able to increase when rare). This reveals that the resident can increase when rare as long as $\alpha_{r,\,away} > \alpha_{i,\,home}$. Thus the invader can displace the resident in our simulations when $\alpha_{i,\,home} > 0.4$. Notice that, this requires $\alpha_{i,\,away} > 0.8$ if the invader possesses the same negative feedback as the resident ($\alpha_{i,\,home} = 0.5\ \alpha_{i,\,away}$); however if the invader does not possess a negative feedback, displacement of the resident occurs when $\alpha_{i,\,away} > 0.4$ (Fig. 1c).

Invasion into more diverse communities

It is clear from above that monocultures are particularly susceptible to invasion because all sites are home sites for the resident. However, if the resident community contains more than one species, each suffering from its own specialist soil pathogens, then a greater fraction of newly-vacated sites are away sites for each of the resident species. To evaluate the effect of resident species diversity (D) on invasion success, we simulated models containing 1, 2, 4, 8 and 16 resident species. In each case, we assumed that all resident species have exactly the same competitive ability on away soils: $\alpha_{r,\,away} = 0.4$, and that they all suffer a negative soil feedback of the same magnitude: $\alpha_{r,\,home} = 0.2$. We further assume that each resident species has its own unique soil pathogens; thus, for each resident species, sites formerly occupied by any other resident species are classified as away sites. This strongly stabilizes dynamics such that residents would coexist indefinitely and at identical abundances were it not for the finite community size.

In our multi-resident simulations, all resident species begin at equal abundance, and are given 500 generations of dynamics prior to the introduction of the invader to 16 sites taken equally from among the resident species. We varied the competitive ability of the invader on away soils over the interval 0.20 to 0.50 in steps of 0.025. We use a narrower interval with finer gradations than in the previous simulations, as it is clear that once $\alpha_{i,\,away} > 0.4$ the invader is a better competitor than all members of the resident community ($\alpha_{i,\,away} > \alpha_{r,\,home}$). We only consider the case where the invader also suffers

from a negative soil feedback, which again, is identical in magnitude to that of the residents ($\alpha_{i,\ home} = 0.5\ \alpha_{i,\ away}$).

As hypothesized, increasing resident diversity increases the minimum competitive ability required for successful invasion (Fig. 2a), increases the time-scale of the invasion (Fig. 2b) and reduces the population size of successful invaders (Fig. 2c). The minimum competitive ability on resident soils required for successful invasion increases asymptotically with increasing resident diversity (Fig. 3a), so the largest change is seen when moving from a monoculture to a two-species mixture and each additional resident species has an increasingly small effect (we demonstrate this point analytically below). We therefore predict that weaker competitors are precluded from invading more diverse mixtures, even though they can invade monocultures of all the constituent species. For any given invader, the final population size also declines monotonically with increasing resident species diversity (Fig. 3b), although the time to reach 1000 individuals (the growth rate when rare) increases linearly with species diversity (Fig. 3c). Thus, the different components of invasion success scale differently with increasing resident diversity.

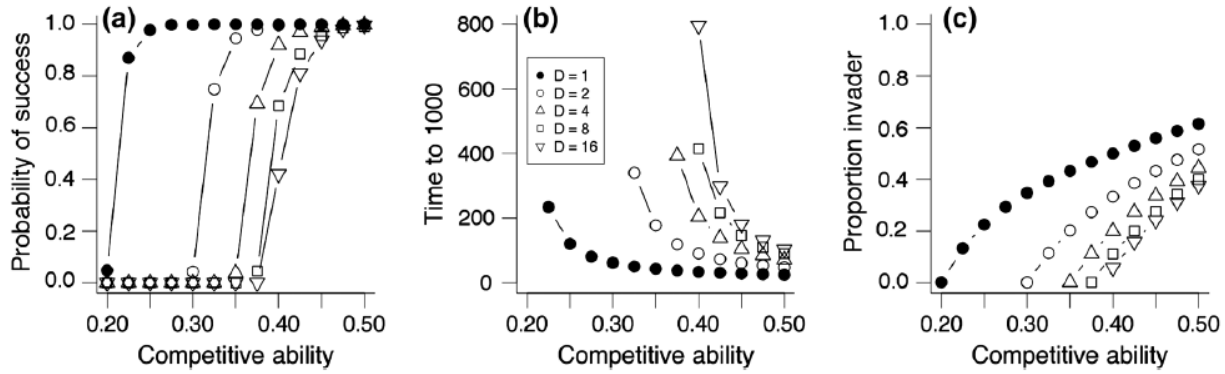

Figure 2. Success of an invader introduced into resident communities of different species diversity (D = 1, 2, 4, 8, 16). The resident species always have the same competitive ability on away soils (0.4) and on home soils (0.2). The probability that the invasion succeeds (a) the time-scale of successful invasion (b) and the final abundance of the invader (c) are shown.

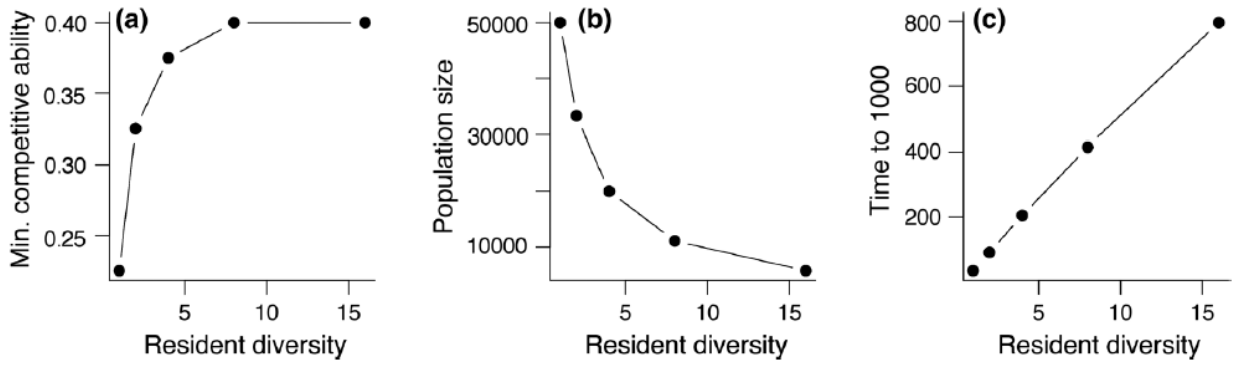

Figure 3. The relationship between resident diversity and the minimum competitive ability required for successful invasion (a). For an invader with exactly the same properties as the resident species, the population size of the invader (b) and the time required to reach 1000 individuals (c) are also shown.

The effects of resident species diversity on invasion success can be shown analytically. If the community contains D resident species, each with identical competitive abilities on home and away soils, then resident species on average have equal abundance; hence each resident species will hold 1/D of the sites not occupied by the invader. Thus, on newly-vacated resident sites, 1/D of the colonizing residents have competitive ability given by $\alpha_{r,\,home}$ and (D-1)/D of the colonizing residents have competitive ability given by $\alpha_{r,\,away}$. We thus replace the resident performance in the second term of the bracketed sum in Eq. 1 with the following:

$$(1-p_{i,t})\left[\frac{\alpha_{i,away}}{\alpha_{i,away}p_{i,t}+\left(\frac{1}{D}\right)(1-p_{i,t})\alpha_{r,home}+\left(\frac{D-1}{D}\right)(1-p_{i,t})\alpha_{r,away}}\right] \quad (5)$$

The condition for the invader to increase when rare now becomes:

$$\alpha_{i,away} > \alpha_{r,away}\left(\frac{D-1}{D}\right)+\alpha_{r,home}\frac{1}{D} \quad (6)$$

Condition 6 reveals that the invader's competitive ability on away soils must exceed a weighted average of the residents' competitive abilities on home and away soils. With D = 1 (a monoculture), the first term of the sum disappears and we return to condition 4. As diversity (D) increases, the second term of the sum decreases, and because $\alpha_{r,\ away} > \alpha_{r,\ home}$, invasion becomes more difficult. Also notice that the greatest decrease in the weighting of $\alpha_{r,\ home}$ (and hence the greatest change in invasion resistance) occurs when D goes from 1 to 2, matching simulations in Fig. 2a and 2b.

Discussion

The loss of natural enemies has often been implicated in the success of exotic species in their introduced range (Keane and Crawley 2002) while the inevitable corollary – that plant populations normally experience natural enemy regulation in their native range – has been largely overlooked. One possible general mechanism for this regulation is negative plant-soil feedbacks, where species are disadvantaged on previously-occupied or home sites, analogous to the Janzen-Connell effect. There is widespread empirical evidence for species-specific negative soil feedbacks within plant communities, including evidence that exotic species experience weaker negative feedbacks than native species (Bever 1994, van der Putten and Peters 1997, Klironomos 2002, Bartelt-Ryser et al. 2005, Bonanomi et al. 2005). Several authors have therefore suggested that release from negative feedbacks may allow species to become invasive (van Grunsven et al. 2007, Engelkes et al. 2008).

We used a simple modelling approach to evaluate the potential benefit of escaping regulation by specialist soil pathogens. We found that the loss of a negative soil feedback has no influence on whether or not a given species is able to invade a resident community. Freedom from negative soil feedbacks alone cannot, therefore, allow a species to expand its range or enter new communities. Instead, the probability of successful invasion depends strongly on the competitive ability of the invader on resident soils. This is because, at the start of an invasion, the invader is at low density and so it mainly encounters resident sites. While limited dispersal inevitably changes this outcome to some degree (Bolker and Pacala 1999, Eppstein and Molofsky 2007), it seems unlikely that an invader will be successful if it cannot compete strongly against

the residents on resident soils. Invader competitive ability on resident soils is also the primary determinant of the invader growth rate when rare; hence increased competitive ability on away sites also leads to more rapid invasion.

In contrast, if all resident species are affected by a generalist soil pathogen to which an invader is immune (a different form of enemy release), then this could give an invader a competitive advantage on resident soils. Hence, this type of enemy release could increase the probability of invasion success; however, in this case the invader has become successful, not through the loss of regulation, but rather because it has acquired a large fitness differential with respect to resident competitors. Such an effect would be better quantified by comparing the competitive ability of residents versus the invader on sterilised and non-sterilised soil from the introduced range. Notice that in this case we are assigning a rather different role to pathogens in native communities, instead of being species-specific and providing regulation and stabilisation, they have an equalising role by having a general negative effect on all residents (Chesson 2000). A similar effect is proposed to occur if species lose specialist pathogens or herbivores and as a result are able to evolve increased competitive ability – the EICA hypothesis (Blossey and Notzold 1995).

Invaders freed from negative soil feedbacks tend to achieve higher population densities once successful and are more likely to exclude resident species (Levine et al. 2006, Eppstein and Molofsky 2007). Exclusion of residents is still possible when the invader possesses pathogen regulation, should the invader compete strongly enough on all soil types; however, without pathogen regulation, the invader can reach higher abundance than the resident despite being a poorer competitor on away soils. Comparisons of performance on ‘average’ soil, compost mixes, or even the invader soil, would therefore be unrevealing. This emphasises the need for carefully controlled experiments on different soil types. Notice that although we used a strong feedback for our simulations, weak negative feedbacks give qualitatively similar results.

Monocultures of resident species which suffer negative soil feedbacks are particularly susceptible to invasion. In a monoculture, all sites are home sites for the resident, reducing the resident's average competitive ability across all available sites. Increasing resident diversity increases community resistance because many of the

available sites are now away sites for each of the resident species. This raises the collective competitive ability of the resident community and makes the system more difficult to invade. Increasing resident diversity has diminishing returns; an asymptotic relationship emerged between resident diversity and invader establishment probability. In our models, resident species have identical characteristics, so that the increased invasion resistance of diverse communities is not due to a 'selection' effect by which mixtures are more likely to include species with particularly high resistance to invaders (Loreau and Hector 2001). Thus, a species which cannot invade a diverse mixture could potentially invade monocultures of all the constituent species.

The literature on invasion success in experimental manipulations of biodiversity reveals several patterns consistent with our results. First, the number of invading species and total invader biomass decrease with resident diversity in a non-linear way (Tilman 1997, Knops et al. 1999, Naeem et al. 2000, Hector et al. 2001, Fargione et al. 2003) and second, several biodiversity experiments report that monoculture performance often declines with time (Pfisterer et al. 2004, Fargione et al. 2007). Although pathogens have not been directly implicated in either of these results, our models suggest that they could play some role. Many biodiversity experiments also reveal that functional group diversity is as important as species diversity in determining ecosystem functioning (Tilman et al. 1997, Hector et al. 1999, Hooper and Dukes 2004, Spehn et al. 2005). Although our models assumed that species possess unique pathogens, such results could be explained if species within the same functional or taxonomic group share pathogens (De Deyn et al. 2003, Gilbert and Webb 2007). For example, the increasing success of phylogenetic distance in explaining a variety of ecosystem performance measures (Cadotte et al. 2008) could also be attributed to pathogens that cross-infect closely-related hosts, as could the observation that species are more successful in invading communities from which their own functional group is absent (Fargione et al. 2003, Turnbull et al. 2005).

Conclusion

Plant ecologists have tended to overlook natural enemies in favour of resource-based explanations for community dynamics and structure (Harpole and Tilman 2007). However, a considerable body of evidence now demonstrates that pathogens, although often unseen, can have large and predictable effects on resident fitness (van der Heijden et al. 2008). Negative soil feedbacks in particular can act in a frequency-dependant manner to promote diversity (Bever 2003) and, as we have shown here, could also endow diverse pathogen-regulated communities with increased invasion resistance.

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Chapter 7

Biology, chance or history? The predictable re-assembly temperate grassland communities.

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Abstract

Many studies have examined invasion resistance in plant communities, but few have explored the mechanisms of invasion and how subsequent community reassembly affects community functioning. Using natural dispersal and deliberate seed addition into grassland communities with different compositional and richness histories, we show that invaders establish in a nonrandom manner due to negative effects of resident functional groups on invading species from the same functional group. Invaders hence complement communities with originally low richness levels. Consequently, communities converge toward similar levels of species richness, high functional richness, and evenness, but not always maximum productivity. Invasion processes are faster but qualitatively similar when the effect of chance, in the form of dispersal stochasticity, is reduced by seed addition. Thus, dispersal limitation may influence community assembly, but it does not override functionally predictable assembly mechanisms. Some of the most productive communities prior to invasion are unstable in the face of invasion, leading to decreased productivity following invasion. We suggest that invasion into such communities occurs possibly because a pathogen-free niche is available rather than a resource niche. Thus, pathogens in addition to resource niches may be important biological drivers of community assembly.

Introduction

Biology, chance, and history must all play some role in community assembly. For example, in order to successfully establish in a new community, a potential invader must first arrive, and dispersal is an inherently stochastic process. However, the relative importance of dispersal limitation and historical contingency vs. deterministic biological interactions is still hotly debated (e.g., Drake 1991, Hubbell 2001, Chase 2003, Fargione et al. 2003, Turnbull et al. 2005a, b).

The first explanations as to why certain species were able to successfully invade new communities were certainly deterministic in nature and focussed mainly on the biology of the invaders (see, e.g., Elton 1958). For instance, some species appeared to be more successful than others at dispersing to new sites, at entering new communities, or at reaching high population sizes and suppressing residents (Crawley 1986, Drake et

al. 1989). This observation led to a focus on the properties of these species and their associated “invasiveness” (Baker 1967, Sutherland 2004, Richardson and Pysek 2006).

Conversely, invasion success might be related to the biology of the invaded or resident community; for example, more diverse communities tend to be more invasion resistant (Crawley 1987, Burke and Grime 1996). This may occur because particular resident species or functional groups provide invasion resistance (Crawley et al. 1999, Levine and D'Antonio 1999, Symstad 2000, Hector et al. 2001, Dukes 2002, van Ruijven et al. 2003, Fargione and Tilman 2005) and these species or functional groups are more likely to be found in higher-diversity communities. The importance of particular species for community invasion resistance is therefore analogous to a sampling effect in biodiversity–productivity relationships (Hector et al. 2001, Wardle 2001).

Finally, interactions between the invader and the invaded community might be key to understanding invasion success, analogous to a complementarity effect in biodiversity–productivity relationships (Hector et al. 2001, Fargione et al. 2003). In this case, not only the identity of the invader or the composition of the resident community, but the match between invaders and communities plus the respective species abundances would be most important in determining the outcome of invasion (e.g., Fargione et al. 2003, Turnbull et al. 2005b, Strauss et al. 2006). Thus, just like species coexistence in established communities, invasion and community reassembly would be controlled by density-dependent stabilizing mechanisms (Chesson 2000). These stabilizing mechanisms would be expected to facilitate invasion by species or functional groups that are most different from abundant residents (MacArthur and Levins 1967, Abrams 1983, Emery 2007).

The most well-known and studied complementarity mechanism within temperate communities is based on resource-use niches (e.g., Harpole and Tilman 2007), which could lead to preferential invasion by species with complementary resource requirements compared with the residents (Fargione et al. 2003, Questad and Foster 2008). Increased invasion resistance of species-rich communities could, according to this hypothesis, be attributed to the lack of unconsumed resources, as some invasion studies have indicated (e.g., Knops et al. 1999, Hector et al. 2001, Fargione et al. 2003). Another stabilizing mechanism potentially underlying invasion patterns is the

presence of pathogens or herbivores—for which the invader is a host or resource—in a community that contains species closely related to the invader. This mechanism is similar to the Janzen-Connell effect, in which the presence of adult trees reduces the recruitment success of conspecific juveniles in tropical forests (Janzen 1970, Connell 1971, Augspurger and Kelly 1984). We have previously found evidence for this mechanism, operating via negative soil feedbacks, in a temperate grassland community where it was a powerful promoter of coexistence between competing functional groups (Petermann et al. 2008). Hence, this pathogen-driven feedback could similarly affect invasion patterns and community reassembly after invasion. Because functional groups are based on species traits, taxonomy, or both (for details regarding the functional-group classification in this paper, see Methods: Experimental design, below), we expect species within functional groups to share more pests and pathogens (Gilbert and Webb 2007) and to have more similar resource requirements and resource-use patterns (Fargione et al. 2003). If invasion and community assembly are driven by one of these two stabilizing mechanisms, between-functional-group effects would be expected to be stronger than within-functional-group effects.

In contrast to these deterministic explanations, invasion and community assembly could be independent of the biology of the species and instead be strongly influenced by chance (Hubbell and Foster 1986, Hubbell 2001). If invasion into new communities is viewed in the light of island-biogeographic theory (MacArthur and Wilson 1963, 1967) the probability of colonization by new species inevitably decreases with increasing species richness of the resident community because a larger fraction of the total species pool has already arrived and established. Thus, a negative relationship between community richness and the number of invading species would be expected. At the same time, the number of species going extinct is predicted to increase with increasing resident species richness, as, for the same area, population sizes are smaller in diverse communities. Equilibrium richness is reached when extinction and colonization rates become equal. Under this neutral scenario, the compositions of the assembling communities would be random, meaning that they are not predictable based on the biology of the species, but instead governed only by demographic and dispersal stochasticity (Hubbell 2001). In the case of established communities of different initial

richness and composition, invasion of new species and subsequent community reassembly would then lead to the convergence of species richness but not of composition, even under identical environmental conditions (Fukami et al. 2005). This was indeed found by two recent studies examining spontaneous invasion via natural dispersal into experimental grassland communities of originally different richness levels and compositions (Pfisterer et al. 2004, Rixen et al. 2008). Species have often been shown to be limited by their dispersal abilities (Turnbull et al. 2000, Clark et al. 2007), and propagule pressure has been identified as a major driver of invasion and community assembly (e.g., Kolar and Lodge 2001). Thus, the compositional divergence of different communities observed in spontaneous-invasion studies may well be due to dispersal stochasticity. On the other hand, initial floristic composition (Egler 1954, Collins et al. 1995) or the order of species arrivals (Drake 1990, Chase 2003, Zhang and Zhang 2007) may prevent compositional convergence. In that case, the communities‘ colonization and establishment history may override all other assembly mechanisms and may have a dominant influence on the final composition of reassembled communities (Drake 1991).

The functioning of plant communities, for example in terms of primary productivity, has been found to be a function of species richness (Tilman et al. 1996, 2001, Hector et al. 1999), phylogenetic diversity (Cadotte et al. 2008), functional richness (Tilman et al. 1997, Hector et al. 1999), evenness (Wilsey and Potvin 2000, Polley et al. 2003, Hillebrand et al. 2008), and composition (Hooper and Vitousek 1997, Tilman et al. 1997, Spehn et al. 2005; for further references see Balvanera et al. [2006]). Therefore, if invasion leads to changes in these properties, it is expected to directly or indirectly influence community functioning (Chase 2003, Hooper et al. 2005). However, the consequences of invasion for the invaded communities, especially with regard to their functioning, are rarely considered (Pfisterer et al. 2004, Rixen et al. 2008).

In the present study, we use an established grassland biodiversity experiment with a species richness and functional-group richness gradient maintained by weeding to study the reassembly of communities by invasion and the resulting effects on ecosystem functioning. After opening communities with different initial compositions to

spontaneous invasion and to invasion assisted by seed addition, we examine whether invasion and reassembly processes are dominated by the biological characteristics of residents or invaders, by the chance effects of dispersal, or by the compositional history of the resident community. Furthermore, we assess the consequence of invasion, not only for richness and composition but also for the functioning of reassembled communities in terms of primary productivity. We show that invasion is biologically predictable on a functional-group basis and only weakly dependent on dispersal effects. Invasion complements species richness and functional composition and thus leads to the decay of positive species richness–productivity relationships. We suggest that the observed community reassembly processes were driven by both resource complementarity and pathogen effects.

Methods

Experimental design

The present study was carried out within a large experimental platform at Jena, Germany (50°55′ N, 11°35′ E). The Jena Experiment is a long-term grassland biodiversity–ecosystem functioning experiment (Roscher et al. 2004). It is situated in the floodplain of the river Saale at an altitude of 130 m above sea level and until 2001 it was used for agricultural crops. The experimental grassland plots were established by sowing in spring 2002. The mean annual air temperature is 9.3°C; the mean annual precipitation is 587 mm.

Seventy-eight experimental plots were sown with randomly assembled species assemblages of 1, 2, 4, 8, or 16 species. The total species pool of the experiment consisted of 60 native central European plant species common in seminatural grasslands. Four plots containing all 60 species were also sown. Prior to assembling experimental communities, the species were grouped into four functional groups according to a cluster analysis using ecological and morphological traits (16 grasses, 12 legumes, 12 small herbs, 20 tall herbs; Roscher et al. 2004). Each functional group was represented at each richness level. In addition, the number of functional groups was varied within species-richness levels as much as possible, including 16 species-richness levels with only one functional group, so that the design was almost completely

orthogonal with respect to functional-group composition and species richness (Roscher et al. 2004). There were 16 different species in monoculture; 16 different species compositions at richness levels 2, 4, and 8; and 14 different species compositions at richness level 16 (see Appendix C: Table C1). The plots had a size of 20 × 20 m and were arranged in four blocks. In addition, each plot was assigned x- and y-coordinates to account for geographical position in later analyses. All plots were mown twice a year and did not receive fertilizer.

Within each plot, we marked four 2 × 2.25 m subplots for our invasion experiment (see Plate 1). One pair of subplots was used for the invasion treatment "cessation of weeding" (C) and one pair for the treatment "weeding" (W). In each subplot pair, one subplot was randomly assigned to the deliberate seed-addition treatment (+), and the other received only spontaneous-invader seeds (−). The seed-addition treatment included seeds of all species from the original experimental pool of 60 species and we therefore refer to them as "internal invaders" if they are not part of the sown community of a specific plot. Seeds were added at a rate of 1000 viable (according to standard laboratory tests) seeds/m2 in April 2005 divided equally among the 60 species. Among the spontaneously (= naturally) invading species there were both "internal invaders" and "external invaders," the latter not belonging to the original pool of 60 species but occurring in the surroundings of the field site. Thus, our experimental design consisted of the following four subplots: subplot "W−" was weeded twice a year like the remainder of the larger 20 × 20 m plot to maintain the original set of species ("residents") and served as the control ("closed" community). In subplot "W+" internal invader seeds were added and external invader species were removed by weeding, so that only internal invaders could establish. In subplot "C−" weeding was stopped at the end of 2004; hence, internal invaders and external invaders could enter the community spontaneously. In subplot "C+" weeding was also stopped at the end of 2004, so that internal and external species could invade spontaneously; additionally, internal-invader seeds were added. Generally, soil disturbance caused by weeding was kept to a minimum by using small knives to cut weed roots and remove them carefully and by all maintenance being done before the development of a closed canopy (early April at the start of the growing season, and July after the first mowing).

Plate 1. Three main plots (20 × 20 m) of the Jena Experiment in the foreground, with the invasion subplots discernible by the conspicuous white flowers of invading oxeye daisy (Leucanthemum vulgare). Photo credit: Forschergruppe—the Jena Experiment.

We harvested aboveground plant biomass (above 3 cm) twice a year for three years after the start of the invasion experiment, i.e., from year 4–6 after the initial establishment of the plots. Harvests were timed to coincide with typical grassland harvest times in central Europe (late May and August). In each subplot we randomly selected an area 20 × 50 cm for harvest. We sorted the harvested plant material into species, except in the first of the two harvests in 2005, when we only sorted into residents, internal invaders, and external invaders, and noted the number of species in each category. Harvested biomass was dried and weighed. Comparative data from weeded monocultures of all 60 species and weeded 60-species mixtures were available from another study within the Jena Experiment (Marquand et al. 2009).

Data analysis

We analyzed the biomass and the number of species of residents and internal and external invaders as a function of the design variables and covariates with ordinary mixed-model analyses of variance (Snedecor and Cochran 1980). Fixed and random terms were fitted sequentially by multiple regression and results summarized in analysis of variance (ANOVA) tables (for more details, see Schmid et al. [2002]). Biomass (in g/m2) was analyzed as a yearly total, and species richness (per harvest quadrat) as an average of the two harvests per year. Because sown resident-species richness in the plots was highly correlated with realized resident-species richness in the harvested area at the start of our experiment, we used sown plot richness in all analyses that investigate the influence of preinvasion community properties on invasion. Results did not change when realized richness was used. The number of internal invader species and their biomass was analyzed on a functional-group basis in a "home–away" contrast analysis. This allowed a test of the difference in invasion success between communities where each functional group occurred among the residents ("home") and where it did not ("away"). In the home–away biomass analysis we included only data from 2006 and 2007, as the biomass of individual functional groups was only available for one of the two harvests in 2005.

The first section of this paper focuses on the influence of community properties and invader-species characteristics on invasion success. Therefore, only data from invaded subplots were used (C−, C+, and W+) in the respective analyses. The second section of the paper deals with community changes in response to invasion. Thus, the development of the non-invaded subplot (W−) was compared with invaded subplots that contained the full invader range (external and internal invaders: C− and C+). All analyses that classify invaders by functional group exclude external invaders because the grouping of internal species into functional groups was based on an a priori cluster analysis (see Experimental design, above) and external invaders occurred in very low species numbers and abundance. Data were analyzed using the statistical software R 2.7.2 (R Development Core Team 2008) and GenStat, eleventh edition (VSN International 2008). All error bars and errors accompanying mean values represent ±1 standard error of the mean.

Results

Community invasibility

Following the cessation of the weeding regime, communities of residents accumulated increasing numbers of invader species with time. However, the number and biomass of internal invader species (species that belonged to the species pool of the experiment) and external invader species decreased with increasing resident-species richness, i.e., resistance to invasion increased with resident-species richness (Fig. 1, $F_{1,63} = 80.23$, $P < 0.001$ for the number of internal invader species; $F_{1,63} = 32.03$, $P < 0.001$ for internal-invader biomass; $F_{1,67} = 22.03$, $P < 0.001$ for the number of external invader species; $F_{1,67} = 13.61$, $P < 0.001$ for external-invader biomass; full ANOVAs can be found in Appendix C: Tables C2–C4). For internal invaders, this effect may in part be due to the decrease in the number of potential internal invader species in more diverse plots (MacArthur and Wilson 1967, Hector et al. 2001). However, this cannot apply to external invaders because their number is not intrinsically related to the number of resident species. Because the biomass of the resident community increased with sown species richness, we tested its direct effect on invader success by including resident biomass as a covariate in the analysis. Resident biomass had a strong negative effect on the number and biomass of internal and external invader species ($F_{1,920} = 106.20$, $P < 0.001$ for the number of internal invader species; $F_{1,920} = 514.27$, $P < 0.001$ for internal-invader biomass; $F_{1,160} = 79.32$, $P < 0.001$ for the number of external invader species; $F_{1,160} = 10.36$, $P = 0.002$ for external-invader biomass). Nevertheless, the inclusion of resident biomass as a covariate did not affect the significance of subsequent terms in the ANOVA, indicating that resident biomass effects were additive to the other effects.

Fig. 1. (a, b) The number of species and (c, d) the biomass of internal and external invaders as a function of resident-species richness (log scale). The solid lines represent subplots without seed addition, and the dashed lines represent subplots with seed addition (see Methods: Experimental design for details). The data (mean ± SE) were averaged over the six harvests from years 2005–2007. Note the change in the y-axis scale for the internal and external invaders. For statistical analysis, see Appendix C: Table C2.

Invasiveness

Internal invader species were much more successful than external invaders in invading new communities, even if their seeds were not added deliberately. On average, internal invaders made up 85% of all invader species and 95% of total invader biomass (Fig. 1). Compared with the spontaneous-invasion treatment, the deliberate addition of seeds of internal invaders further increased the number of successfully invading internal species when resident species richness was low (Fig. 1a, $F_{1,596} = 47.44$, $P < 0.001$ for the

interaction “Species richness × Seed addition”) and increased internal-invader biomass at all species-richness levels (Fig. 1c, $F_{1,595} = 8.4$, $P = 0.004$ for the term “Seed addition”). External invaders were neither negatively nor positively affected by the experimental addition of seeds of internal species (Fig. 1b, d, $F_{1,75} = 2.47$, $P = 0.120$ for the number of external invader species; and $F_{1,75} = 0.80$, $P = 0.375$ for external-invader biomass). Furthermore, there was no effect of external invaders on invasion success of internal invaders ($F_{1,155} = 0.25$, $P = 0.620$ for the number of external invader species; $F_{1,155} = 0.28$, $P = 0.600$ for external-invader biomass).

Because of the small biomass contribution of external invaders further analyses were carried out only for internal invaders. Among internal invaders, functional groups and species still varied widely in their ability to establish in new communities. The most successful invading functional groups in terms of the number of established species were grasses and small herbs (1.2 ± 0.01 and 1.1 ± 0.01 invader species per harvest quadrat, respectively, vs. 0.6 ± 0.01 legume and 0.6 ± 0.01 tall-herb invader species per quadrat [mean ± SE]). Grass and legume invaders produced the highest biomass (89 ± 4 g/m^2 and 87 ± 4 g/m^2, vs. 57 ± 4 g/m^2 and 35 ± 4 g/m^2 for small-herb and tall-herb invaders, respectively). When all internal invaders were examined separately at the species level, we found that the invasiveness of a species in terms of biomass production in a new community was weakly positively correlated with its aboveground biomass in monoculture ($R^2 = 0.15$, $F_{1,57} = 10.14$, $P = 0.002$) but strongly positively correlated with its aboveground biomass in 60-species mixtures ($R^2 = 0.51$, $F_{1,55} = 55.29$, $P < 0.001$). Thus, the best predictor of invader performance was resident performance of the particular species in highly diverse resident communities.

The success of invader species or functional groups also depended on the interaction between the invader and the resident species in a community. Both the number of internal invader species and their biomass were reduced when the functional group they belonged to was already present among the residents (“home”), compared to when it was absent (“away,” Fig. 2). We analyzed this negative interaction (negative home–away effect) between the same resident and invading functional groups as a separate contrast within all resident and invading functional-group interactions and found it to be significant ($F_{1,11} = 37.94$, $P < 0.001$ for species number; $F_{1,11} = 6.50$, $P =$

0.027 for biomass). Additional interactions between resident and invader functional groups also influenced invader success. However, these other interactions were less important than the negative home–away effect, and the latter was even significant when tested against these other interactions (i.e., the deviation from main contrast). We found a stronger negative home–away effect with seed addition than with spontaneous invasion ($F_{1,11}$ = 20.41, P < 0.001 for the number of species; $F_{1,11}$ = 6.65, P = 0.026 for biomass).

Because we could not distinguish between invader and resident individuals of the same species, species-level home–away effects on invader biomass could not be measured. However, when negative home–away effects on invader biomass at the functional-group level were examined separately for all species, it became apparent that about two thirds of the species experienced these negative home–away effects. They were strongest for species that were generally successful invaders (in terms of biomass production), while species with generally low invasiveness experienced neutral to positive home–away effects (Fig. 2c).

Fig. 2. Negative home–away effect when plants invade communities where their functional group is already present. (a) The number of internal invader species and (b) the internal-invader biomass are shown separated into four functional groups, with paired bars representing plots where the same functional group (FG) is already present with at least one species (white bars, “home”) or where the same FG is not yet present (gray bars, “away”). Data are means ± SE. (c) Log-ratio of the home and away biomass of the internal invaders: log(biomass at home/biomass away). Negative log-ratio values correspond to a disadvantage in a home plot (negative home–away effect); positive log-ratio values indicate a home-plot advantage (positive home–away effect). The effect is based on FG–home and FG–away invasion, but each bar represents a single internal invader species (the number by each bar identifies each of the invader species; for species names see Appendix A). Almost all of the dominant invader species (black bars indicating average biomass in home and away communities >10 g/m2) experience negative home–away effects, whereas subordinate species (white bars indicating average biomass in home and away communities <0.1 g/m2) show mostly positive home–away effects. The data were averaged across the three subplots (across the spontaneous-invasion [C−] and seed-addition [C+ and W+] treatments), across four harvests from 2006–2007, and across species-richness levels (range: 1–16 species). For statistical analysis, see Appendix C: Table C2.

Community convergence through invasion

Species richness, functional richness, and productivity.— Following the cessation of weeding, total species richness of communities with initially low richness experienced a major richness increase while those communities with the highest original species richness showed a slight decrease in species richness, leading to convergence in species richness due to invasion (Fig. 3, $F_{1,224} = 20.98$, $P < 0.001$ for the interaction "Species richness [$\log_2$] × Invasion × Year"; the full ANOVA can be found in Appendix C: Table C5). At the same time, the number of resident species in the weeded controls remained relatively constant. Seed addition caused the total species richness of the invaded communities to increase slightly more rapidly than in communities with spontaneous invasion ($F_{1,224} = 3.02$, $P = 0.084$ for the interaction "Species richness [$\log_2$] × Seed addition × Year"), especially in communities with originally low resident-species richness, and to reach somewhat higher levels at the end of the observation period ($F_{1,224} = 27.46$, $P < 0.001$ for the interaction "Seed addition × Year"). If the lines in Fig. 3a were extended beyond 2007, monocultures and 60-species mixtures were predicted to cross in 2009 at a richness level of 12 species (per harvest quadrat) with only spontaneous invasion, whereas the lines for communities receiving deliberate seed additions (Fig. 3b) were predicted to cross in 2008 at a level of 15 species. This suggests that with the pressure of seed addition, species richness converges more rapidly. The number of resident species remained stable during the invasion phase in all plots except the 16- and 60-species mixtures, where slight decreases over time were observed (data for residents not shown separately). All increases in species richness were entirely due to newly establishing invader species and not to a reinvasion of previously extinct residents.

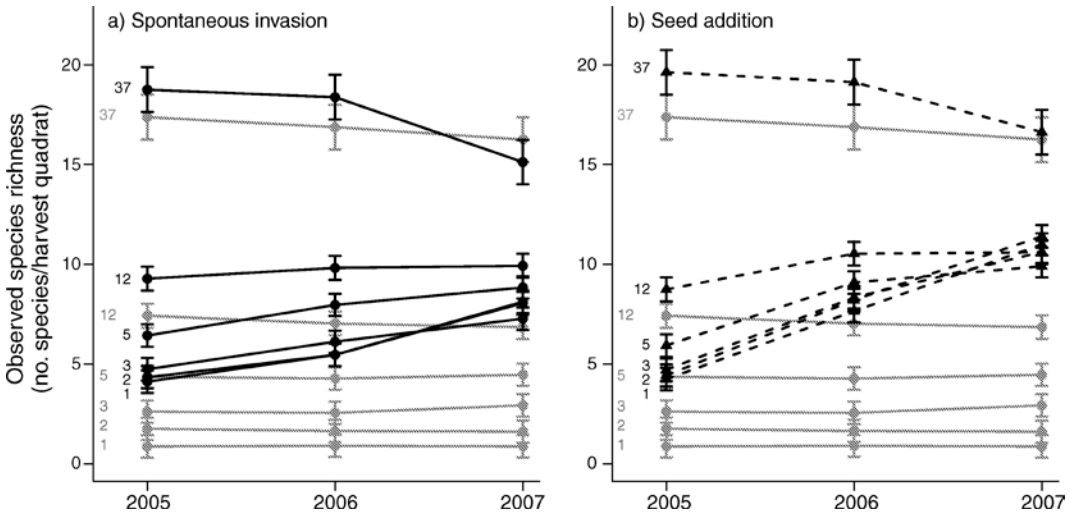

Fig. 3. Convergence of species richness in invaded communities. Note that species richness in weeded controls (gray lines) could only decline because all invaders were weeded out and were therefore not included in harvests. The species richness of invaded communities (black lines) includes residents and invaders: (a) black solid lines show spontaneous invasion (C−); (b) black dashed lines show seed addition (C+). Data are means ± SE. The numbers at the beginning of each line depict the average species richness per 20 × 50 cm harvest quadrat of the respective communities in 2003, prior to the start of the invasion experiment. For statistical analysis, see Appendix C: Table C5.

While species richness had not fully converged by the end of the experiment, functional richness increased rapidly in invaded communities and in the last year of observation, 69 and 77 out of 82 communities in the spontaneous-invasion and seed-addition treatments, respectively, contained all four functional groups, even in the rather small area that was harvested. In contrast, only 12 out of 82 control communities contained all four functional groups in an area of the same size. Shannon diversity indices for functional-group (FG) richness remained low in weeded controls until the end of the observation period (H = 0.55 ± 0.02 if based on the relative number of species in each FG and H = 0.41 ± 0.02 if based on the relative biomass in each FG) but increased in spontaneous-invasion (H = 1.22 ± 0.02 if based on the number of species and H = 0.94 ± 0.02 if based on biomass) and seed-addition treatments (H = 1.30 ± 0.02 if based on the number of species and H = 1.06 ± 0.02 if based on biomass). Not only did invasion lead to high FG richness but also to the convergence of FG proportions to similar levels in previously different communities (Fig. 4 and Appendix B:

Fig. B1). The average composition of the biomass in invaded subplots at the end of the experiment was 30% grasses, 29% legumes, 24% small herbs, and 17% tall herbs, and thus showed very high functional evenness.

Community biomass was much more variable than species richness and functional richness, and this was largely due to the biomass of resident species varying between years. The biomass of invaders increased over the course of the experiment ($F_{1,921} = 154.69$, $P < 0.001$), except in the 60-species mixtures, where it remained close to 0. In general, total community biomass increased from 2005 to 2007 in invaded communities ($F_{1,224} = 16.54$, $P < 0.001$ for the interaction "Invasion × Year") but increased most strongly in communities with originally low resident-species richness. Therefore, communities of different levels of original species richness and hence different community biomass production became more similar following invasion ($F_{1,150} = 14.99$ $P < 0.001$ for the interaction Species richness [$\log_2$] × Invasion).

Biodiversity–productivity relationship.

At the beginning of the experiment in 2005 we found a positive realized species richness–productivity relationship in all subplots (Fig. 5a). This relationship was maintained across the three years in the weeded control subplots (gray lines in Fig. 5). However, in the subplots that were opened to invasion the positive relationship decayed over time (black lines in Fig. 5; Appendix C: Table C6; $F_{1,239} = 5.80$, $P = 0.017$ for the interaction Realized richness [$\log_2$] × Invasion × Year). This decay occurred more rapidly in subplots with deliberate seed addition than in subplots with only spontaneous invasion; the positive relationship had disappeared by 2006 in subplots where invasion was assisted by seed addition, and by 2007 in subplots with spontaneous invasion (Fig. 5).

Fig. 4. Convergence of the proportion of biomass accounted for by the four functional groups. Observed (realized) proportions were calculated as observed biomass of the respective functional group per observed total target biomass. Here, external invaders were excluded because they could not be grouped into the same four functional groups, so target species in this case were residents in weeded controls (gray lines, W−), but residents and internal invaders in non-weeded subplots (black lines in the left column show spontaneous invasion, C−; black dashed lines in the right column show seed addition, C+). Data are means ± SE. Legumes and small herbs were originally sown (2002) in the following proportions: 0, 0.2, 0.25, 0.3125, 0.375, 0.5, and 1. The proportions of tall herbs originally sown were: 0, 0.25, 0.3125, 0.333, 0.375, 0.5, and 1. The proportions for grasses sown were: 0, 0.25, 0.267, 0.3125, 0.375, 0.5, and 1. In 2005 data were only collected in August; in 2006 and 2007 they were collected in both May and August (the average of the harvests is shown).

In contrast to the species richness–productivity relationship, the relationships between the proportion of particular functional groups (based on their realized biomass) and community productivity did not decay due to invasion but rather strengthened (Fig. 6; Appendix C: Table C7). Thus, invaded communities with an above-average proportion of legumes had above-average productivity; and invaded communities with an above-average proportion of small herbs had below-average productivity. The most productive invaded plots (Fig. 6b, c) were originally mainly grass and small-herb monocultures (e.g., Poa pratensis, Festuca pratensis, Bellis perennis, Plantago lanceolata) or non-legume mixtures (e.g., a Plantago media–Taraxacum officinale mixture, and a four-species tall-herb mixture) and had obtained their high, probably unstable, legume proportions via invasion. In contrast, the least productive invaded plots (Fig. 6e, f) were those where small herbs had been present in high proportions from the beginning and had not yet been reduced to the average level of around 24%. Most of these small-herb-dominated communities contained Prunella vulgaris and Ajuga reptans, two small-herb species that can form dense ground cover and can thus slow down invasion by other functional groups. Among the non-invaded communities, plots with 0% or 100% legumes were less productive than others, and plots with 0% small herbs were slightly less productive than those with a small proportion of small herbs.

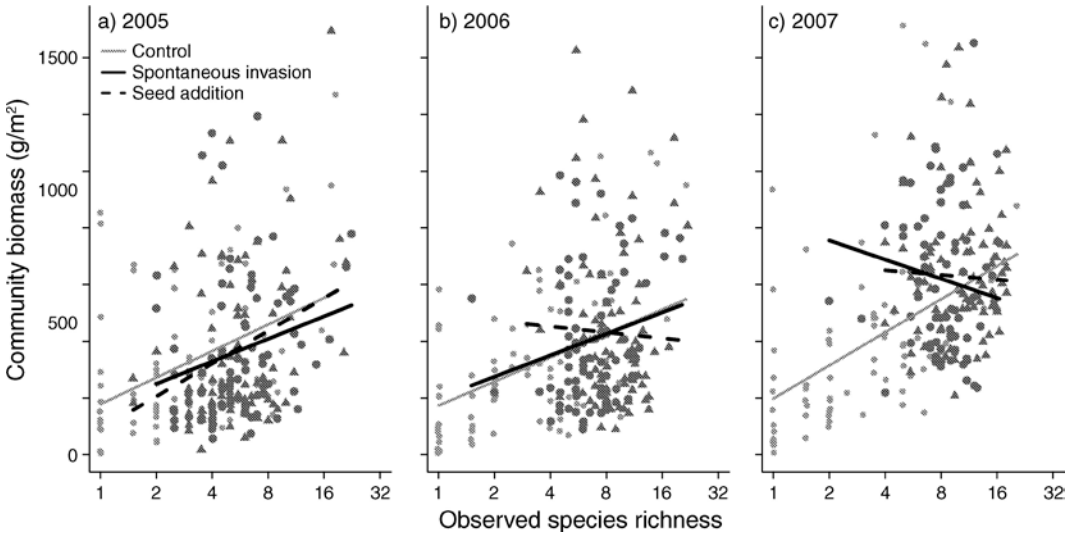

Fig. 5. Observed (realized) species richness–productivity relationships over the course of three years (note y-axis log scale). The light gray line and circles depict the weeded control (no-invaders treatment, W−), the solid black line and dark gray circles depict the spontaneous-invasion treatment (C−), and the dashed line and dark gray triangles depict the seed-addition treatment (C+). Note that regression lines are drawn only across the range of observed species-richness values occurring in that respective treatment (one single outlier in 2006 and two in 2007, all three with very high biomass, are not shown). For statistical analysis, see Appendix C: Table C6.

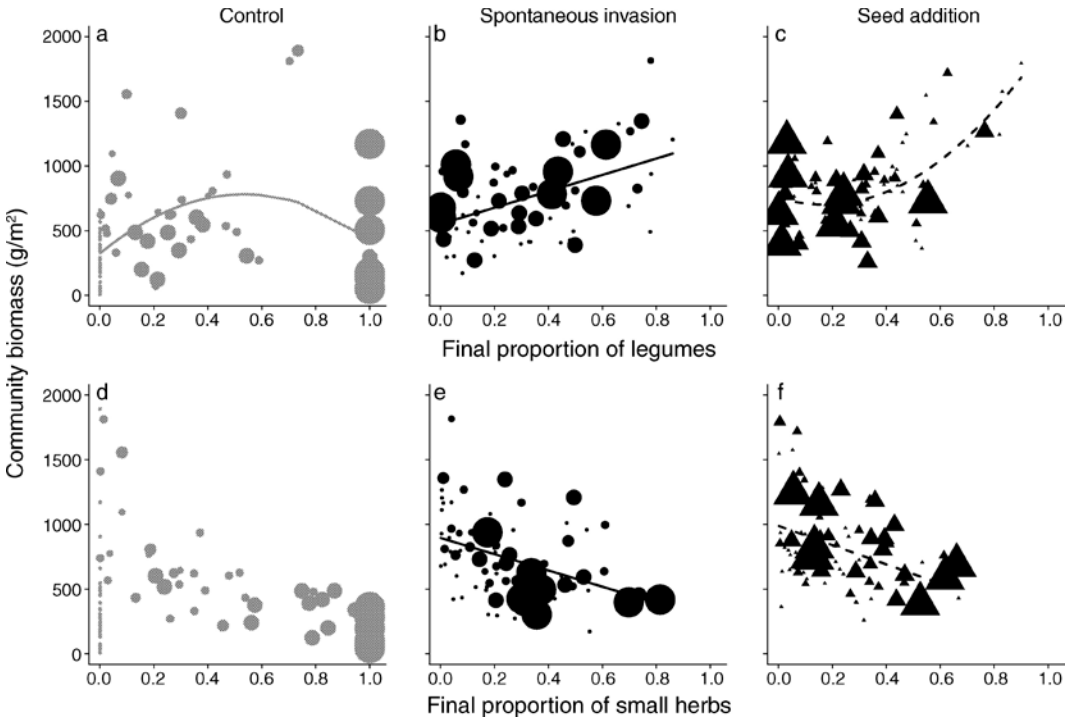

Fig. 6. Legume proportion–productivity relationships and small-herb proportion–productivity relationships in the final year of the experiment: (a,d) control, W−; (b,e) spontaneous-invasion treatment, C−; and (c,f) seed-addition treatment, C+. The plotted symbol size is proportional to the original proportion of the respective functional group in the plot. Note that fitted lines are drawn only when the relationship is significant at $P < 0.05$ and only across the range of realized proportion values occurring in that respective treatment (three outliers with very high biomass were excluded). For statistical analysis, see Appendix C: Table C7.

Discussion

Invasibility and invasiveness

Our experiment confirms previous findings, that experimental communities with higher numbers of resident species are more resistant to invasion from both internal and external invaders than species-poor communities (Tilman 1997, Knops et al. 1999, Joshi et al. 2000, Levine 2000, Naeem et al. 2000, Hector et al. 2001, Kennedy et al. 2002, Fargione et al. 2003, van Ruijven et al. 2003, Pfisterer et al. 2004, Maron and Marler 2008, Roscher et al. 2009a). We also found that the invasion process was highly nonrandom on the functional-group level. Invasion success was partly related to the identity of the invader and to the presence of particular functional groups (e.g., legumes)

in the resident community. However, invasion success was most strongly dependent on the biological difference between the invader and the invaded community (Strauss et al. 2006, Suter et al. 2007), permitting species that belonged to a functional group absent from a community to invade more easily than species belonging to a functional group already present (Fargione et al. 2003, Turnbull et al. 2005b, Mwangi et al. 2007). This strong negative interaction between residents and invaders of the same functional group could be due to overlapping resource requirements (e.g., Knops et al. 1999, Naeem et al. 2000, Fargione et al. 2003, Mwangi et al. 2007) or to the presence of natural enemies (Petermann et al. 2008). We discuss these possibilities in more detail in the next section. However, that the strongest invader species in our study were most strongly inhibited by this negative effect (see Fig. 2c) is supportive of its important role as a stabilizing force in community assembly (Chesson 2000, Chave 2004).

Effects of invasion on community properties and functioning

After our experimental communities were opened to invasion, initially species-poor communities were supplemented with high numbers of invader species. In contrast, originally species-rich communities tended to lose resident species and our experimental communities converged towards species-richness levels very similar to natural grasslands adjacent to our study plots (15–19 species per harvest quadrat). This suggests a shift of the experimentally assembled communities toward naturally assembled communities, at least in terms of species richness, and supports similar findings from invaded grasslands by Pfisterer et al. (2004) and Rixen et al. (2008). However, their studies lacked a weeded control and a seed-addition treatment, and did not analyze whether invaders entered the community in a random or deterministic way. Both studies observed very little compositional convergence suggesting that stochastic effects strongly influenced the reassembly of their communities (but perhaps the short observation time (Pfisterer et al. 2004) or slow plant growth in an alpine habitat (Rixen et al. 2008) also had an influence). In contrast, in the present study nonrandom invasion led to a rapid convergence of functional-group composition among plots and resulted in a high functional richness and evenness of most invaded communities by the end of the experiment. Our experimental communities apparently reassembled toward a common

community structure determined by site conditions. As a consequence of this reassembly, the communities lost their positive species richness–productivity relationship as indicated by previous experiments (Pfisterer et al. 2004, Rixen et al. 2008, Roscher et al. 2009b). Interestingly, observational biodiversity–ecosystem functioning studies within single sites similarly do not find positive species richness–productivity relationships. Thus, our results from reassembled experimental communities help to reconcile apparently contrasting experimental and observational findings (Schmid and Hector 2004, Hector et al. 2007).

In contrast to the rapid decay of the positive species richness–productivity relationship, relationships between functional-group proportions and productivity were maintained or even strengthened in invaded communities. More specifically, invaded communities with a high proportion of legumes produced more biomass, and even outperformed non-invaded communities containing only legumes (for a detailed analysis of functional-group contributions to productivity in non-invaded communities of the Jena Experiment see Marquard et al. 2009). In contrast, invaded (and non-invaded) communities with a high proportion of small herbs produced less biomass than other communities. Some of these unproductive small herb communities proved to be rather resistant to invasion, potentially due to a dense ground cover, representing a historical effect on community structure (Drake 1991). We know from another experiment within the same site that the manual removal of these unproductive species leads to a rapid increase in community biomass even with a loss of species richness (Schmitz 2007).

While invasion by legumes was beneficial for community productivity, communities that initially contained only legumes were not those with a high proportion of legumes after invasion, indicating that high legume proportions in these communities are not sustainable in the longer term and are easily invaded in spite of their high productivity. Indeed, the invasion of legume-only communities by other functional groups sometimes led to decreased productivity of the resulting communities. It could be argued that if nonrandom invasion was mainly due to resource complementarity it should lead to increased community productivity because of the use of otherwise-unconsumed resources by the invader. This argument is valid except for the rather unlikely case that invaders enter the community based on available resources but then

“waste” resources, decreasing community productivity due to their inefficiency. In the case of legumes, unilateral facilitation, i.e., the enhancement of other functional groups by legumes due to their nitrogen-fixing ability (Temperton et al. 2007), would be another explanation for the invasibility by less productive functional groups. It is more likely, however, that pathogen-driven negative feedbacks promoted the nonrandom invasion of all functional groups into plots where pathogens of that specific functional group had not yet accumulated; in other words where their pathogen-free niche was vacant (Turnbull et al. 2005b, Mwangi et al. 2007, Petermann et al. 2008). For legumes in particular, this mechanism is supported by reports on the general instability of experimental legume monocultures, which often suffer from extensive pathogen attack (Pfisterer et al. 2004).

The influence of dispersal limitation

Our seed-addition treatment was intended to reduce the influence of dispersal stochasticity on invasion and community convergence. Indeed, we found that the number of invader species, and, to a smaller extent, invader biomass, was lower in the spontaneous-invasion treatment without experimental seed input. This indicates that even those species that were already present at the site were dispersal limited (Roscher et al. 2009a). Under neutrality, dispersal limitation and the resulting stochasticity in colonization rates are key factors shaping communities (Hubbell 2001, Chase 2003, Chase 2007). With dispersal limitation we would expect a greater stochastic and a smaller deterministic component in the reassembly process (Chase 2007). This was exactly what we found: the deterministic control by functional groups was weaker in plots exposed only to spontaneous seed arrivals and the convergence process slower than when seeds were experimentally added. However, invasion into plots with only spontaneous dispersal was still deterministic on a functional-group basis and led to analogous community convergence in terms of species richness, functional richness, and productivity and to a decay of the species richness–productivity relationship. This supports our conclusion that the deterministic, biological component of community assembly was more important than chance in shaping post-assembly communities, at least in terms of their functional structure. While our experiment was not designed to test species-level determinism, we hypothesize that the nonrandom assembly

mechanisms we observed may still operate among species within functional groups, even if in a less stringent way than among species between functional groups.

By following randomly assembled communities of different species and functional composition for three years after opening them to spontaneous and assisted invasion we have shown that invasion success is strongly controlled by the richness of the community and operates in a biologically predictable way, at least on the functional-group level. Specifically, invasion enhances low species richness and rebalances functional-group composition. Consequently, communities with different richness and compositional histories converge at nearly maximum functional richness and evenness, regardless of dispersal limitation, thus rejecting purely neutral concepts of community assembly. Furthermore, we have shown that the invasion process can lead to reduced productivity because communities of high productivity are not necessarily stable. This suggests a role for pathogens as drivers of community assembly, rather than a full control of floristic compositions by different resource requirements of species. We believe that our results and other work on invasion and assembly within native communities not only contribute to the fundamental understanding of how communities are structured and function, but can also help to direct restoration efforts (Temperton et al. 2004, Funk et al. 2008) and understand, predict, and control nonnative invasions (Shea and Chesson 2002, Funk et al. 2008).

Acknowledgements

We thank U. Wehmeier and A. Oswald for their support with the establishment and maintenance of the invasion experiment, all gardeners and helpers for their assistance in the field, and Jasmin Joshi and two anonymous reviewers for helpful comments on the manuscript. The Jena Experiment is funded by the German Research Foundation (FOR 456) and supported by the Friedrich Schiller University of Jena and the Max Planck Institute for Biogeochemistry, Jena. Additional support was provided by the Swiss National Science Foundation (grant number 31-65224-01 to B. Schmid).

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Appendix A. List of internal invader species with corresponding numbers as in Fig. 2c.

1: Trifolium hybridum, 2: Onobrychis viciifolia, 3: Lotus corniculatus, 4: Vicia angustifolia, 5: Lathyrus pratensis, 6: Dactylis glomerata, 7: Medicago lupulina, 8: Vicia cracca, 9: Trifolium pratense, 10: Festuca pratensis, 11: Ranunculus acris, 12: Trifolium repens, 13: Alopecurus pratensis, 14: Trifolium dubium, 15: Phleum pratense, 16: Poa trivialis, 17: Bromus hordeaceus, 18: Prunella vulgaris, 19: Crepis biennis, 20: Trifolium campestre, 21: Centaurea jacea, 22: Festuca rubra, 23: Arrhenatherum elatius, 24: Campanula patula, 25: Poa pratensis, 26: Primula veris, 27: Cirsium oleraceum, 28: Knautia arvensis, 29: Taraxacum officinale, 30: Plantago media, 31: Veronica chamaedrys, 32: Plantago lanceolata, 33: Galium mollugo, 34: Tragopogon pratensis, 35: Rumex acetosa, 36: Anthriscus sylvestris, 37: Leucanthemum vulgare, 38: Achillea millefolium, 39: Avenula pubescens, 40: Pimpinella major, 41: Geranium pratense, 42: Pastinaca sativa, 43: Leontodon hispidus, 44: Trifolium fragiferum, 45: Medicago varia, 46: Ranunculus repens, 47: Leontodon autumnalis, 48: Trisetum flavescens, 49: Glechoma hederacea, 50: Daucus carota, 51: Bellis perenne, 52: Ajuga reptans, 53: Bromus erectus, 54: Anthoxanthum odoratum, 55: Holcus lanatus, 56: Cardamine pratensis, 57: Carum carvi.

The following species are missing from the graph because their average biomass as invaders at home or away was zero and hence no home-away effect could be calculated: Luzula campestris, Cynosurus cristatus, Heracleum sphondylium, Sanguisorba officinalis. The external species Vicia angustifoila (number 4) was accidentally sown as an internal invader into all subplots with seed addition and therefore treated as an internal invader in all analyses. Nomenclature follows Rothmaler (2002).

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Appendix B. A figure depicting convergence of the proportion of the total number of species accounted for by the four functional groups.

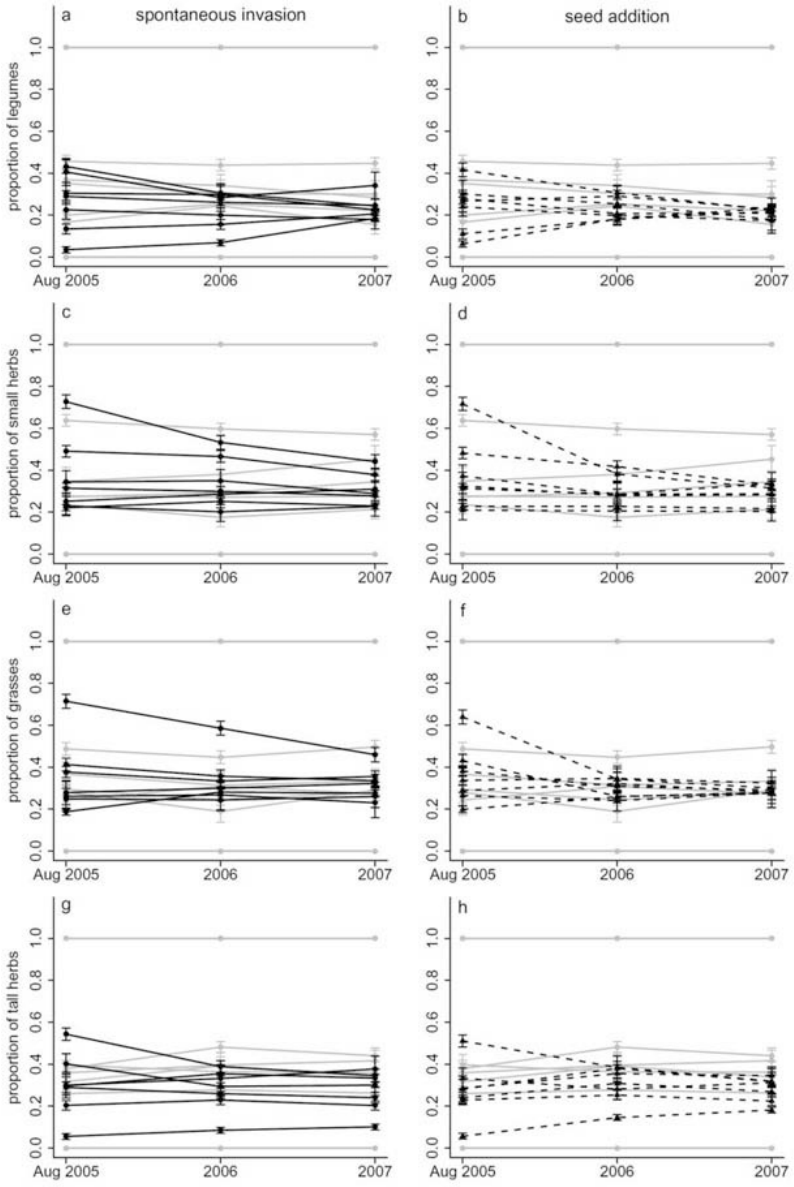

Fig. B1. Convergence of the proportion of the total number of species accounted for by the four functional groups. Observed (= realized) proportions were calculated as observed number of species of the respective functional group per observed number of total target species. Here, external invaders were excluded because they could not be grouped into the same four functional groups, so target species in this case were residents in weeded controls (gray lines, w-), but residents and internal invaders in non-weeded subplots (black lines in the left column: spontaneous invasion c-, black dashed lines in the right column: seed addition c+). Small herbs and legumes were originally sown in the following proportions in 2002: 0, 0.2, 0.25, 0.3125, 0.375, 0.5 and 1, tall herbs: 0, 0.25, 0.3125, 0.333, 0.375, 0.5 and 1 and grasses: 0, 0.25, 0.267, 0.3125, 0.375, 0.5 and 1.

Appendix C. One table showing the experimental design and six tables providing the results of statistical analyses.

Table C1. Experimental design. Original (sown) species richnesss and functional-group composition of resident communities in the Jena Experiment.

Species richnesss	**1**	**1**	**1**	**1**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**
grasses	1				2				1		1		4				2		2		2	1	1		1
small herbs		1				2			1			1		4			2			2	1		1	2	1
tall herbs			1				2			1	1				4			2	2		1	2		1	1
legumes				1				2		1		1				4		2		2		1	2	1	1
Replicates	4	4	4	4	2	2	2	2	2	2	2	2	1	1	1	1	1	1	1	1	1	1	1	1	4
Species richnesss	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**16**	**16**	**16**	**16**	**16**	**16**	**16**	**16**	**16**	**16**	**16**	**60**
grasses	8				4		4		2	3	3		2	16		8		8		5	5	6		4	16
small herbs		8			4			4	3		3	2	2			8			8	6		5	6	4	12
tall herbs			8			4	4		3	2		3	2		16		8	8		6	5		5	4	20
legumes				8		4		4		3	2	3	2				8		8		6	5	5	4	12
Replicates	1	1	1	1	1	1	1	1	1	1	1	1	4	1	1	1	1	1	1	1	1	1	1	4	4

Table C2. Analysis of variance (ANOVA) of the number of species and the biomass (gm^{-2}) of internal invaders per functional group per harvest quadrat. Data exclude the 60-species level, because it always contains all four functional groups. The inclusion of resident biomass as a covariable did not change the significance of the results and was omitted from the model. "Species richnesss" stands for the log_2-transformed sown species richnesss of the resident community. The deviation of the species richnesss effect from log-linearity was not significant and was omitted. The results were relatively robust to the order of the terms species richnesss and presence of particular functional groups, so only the results from the model with species richnesss tested first are shown. The presence of the four functional groups was tested in the order of their explanatory power in the model. Two- and three-way interactions of functional group effects were small and therefore were omitted from the model. The weeding and seed-addition treatments (c-, c+, and w+) were used to form two contrasts. Only the first of them ("Seed addition"), representing seed addition (c+ and w+) vs. no seed-addition treatments (c-), was included in the model (the contrast between w+ and c+ was not significant). The "Home-away contrast", which represents the main contrast within the total "Invader × resident FG" interactions was tested against its deviation ("Other invader-resident FG interactions"). Other error terms are printed in italics. FG = functional group.

Source	**Number of species**				**Biomass**			
	df	**SS**	***F***	***P***	**df**	**SS**	***F***	***P***
Spatial variation	9	51.1	3.01	0.005	9	619241	2.70	0.010
Species richnesss	1	151.3	80.23	<0.001	1	815930	32.03	<0.001
Legume presence	1	83.7	44.38	<0.001	1	302853	11.89	0.001
Tall herb presence	1	18.7	9.89	0.003	1	85362	3.35	0.072
Grass presence	1	0.1	0.05	0.827	1	31173	1.22	0.273
Small herb presence	1	0.2	0.09	0.771	1	8594	0.34	0.563
Plot	*63*	*118.8*	*1.57*	*0.010*	*63*	*1604762*	*0.75*	*0.907*
Invader FG	3	154.1	42.67	<0.001	3	1602127	15.77	<0.001
Home-away contrast	1	145.8	37.94	<0.001	1	2071215	6.50	0.027
Other invader-resident FG interactions	11	42.3	3.19	<0.001	11	3506087	9.41	<0.001
Species richnesss × Home-away contrast	1	4.5	1.77	0.205	1	15375	0.14	0.710
Species richnesss × Other invader-resident FG interactions	14	35.2	2.09	0.014	14	1493090	3.15	<0.001
Plot × Invader FG	*201*	*242.0*	*2.88*	*<0.001*	*202*	*6841052*	*3.03*	*<0.001*
Seed addition	1	75.4	180.62	<0.001	1	93776	8.40	0.004
Species richnesss × Seed addition	1	19.8	47.44	<0.001	1	15019	1.35	0.246
Legume presence × Seed addition	1	19.6	46.91	<0.001	1	3215	0.29	0.591
Tall herb presence × Seed addition	1	1.1	2.66	0.104	1	1568	0.14	0.708
Grass presence × Seed addition	1	0.1	0.20	0.652	1	15890	1.42	0.233
Small herb presence × Seed addition	1	0.0	0.09	0.770	1	62645	5.61	0.018
Invader FG × Seed addition	3	13.9	11.07	<0.001	3	181883	5.43	<0.001
Home-away contrast × Seed addition	1	13.6	20.41	<0.001	1	119186	6.65	0.026
Other invader-resident FG interactions × Seed addition	11	7.3	1.59	0.097	11	197138	1.61	0.093
Plot × Invader FG × Subplot	*596*	*248.8*	*0.57*	*1.000*	*595*	*6639929*	*0.75*	*1.000*
Year	1	580.2	791.58	<0.001	1	2289985	154.69	<0.001
Plot × Invader FG × Subplot × Year	*901*	*660.4*	*2.49*	*<0.001*				
Residual	*944*	*278.3*			*921*	*13634455*	*0.65*	*1.000*

Table C3. Analysis of variance (ANOVA) of the number of species of external invaders per harvest quadrat. Data exclude the 60-species level. The inclusion of resident biomass as a covariable did not change the significance of the results and was omitted from the model. "Species richnesss" stands for the $\log_2$-transformed sown species-richnesss of the resident community. The deviation of the species richnesss effect from log-linearity was not significant and was omitted. The results were relatively robust to the order of the terms species richnesss and presence of particular functional groups, so only the results from the model with species richnesss tested first are shown. The presence of the four functional groups was tested in the order of their explanatory power in the model. Two- and three-way interactions of functional group effects were small and were therefore omitted from the model. All error terms are printed in italics.

	df	**SS**	***F***	***P***
Spatial variation	9	10.3663	0.88	0.549
Species richnesss	1	28.9082	22.03	<0.001
Legume presence	1	10.9671	8.36	0.005
Small herb presence	1	6.0697	4.63	0.035
Tall herb presence	1	0.0013	0.00	0.975
Grass presence	1	0.2808	0.21	0.645
Plot	*67*	*87.9229*	*4.12*	*<0.001*
Seed addition	1	0.823	2.58	0.112
Species richnesss × Seed addition	1	0.6031	1.89	0.173
Legume presence × Seed addition	1	0.0001	0.00	0.986
Small herb presence × Seed addition	1	1.2136	3.81	0.055
Tall herb presence × Seed addition	1	0.7482	2.35	0.130
Grass presence × Seed addition	1	0.044	0.14	0.711
Plot × Subplot	*76*	*24.2346*	*1.18*	*0.190*
Year	1	2.686	9.97	0.002
Species richnesss × Year	1	1.0648	3.95	0.049
Legume presence × Year	1	2.732	10.14	0.002
Small herb presence × Year	1	2.3659	8.78	0.004
Tall herb presence × Year	1	0.0043	0.02	0.900
Grass presence × Year	1	0.3525	1.31	0.255
Seed addition × Year	1	0.1736	0.64	0.423
Plot × Subplot Year	*155*	*41.7673*	*0.91*	*0.713*
Residual	*160*	*47.1871*		

Table C4. Analysis of variance (ANOVA) of the biomass (gm^{-2}) of external invaders per harvest quadrat. Data exclude the 60-species level. The inclusion of resident biomass as a covariable did not change the significance of the results and was omitted from the model. "Species richnesss" stands for the log_2-transformed sown species richnesss of the resident community. The deviation of the species-richnesss effect from log-linearity was not significant and was omitted. The results were relatively robust to the order of the terms species richnesss and presence of particular functional groups, so only the results from the model with species richnesss tested first are shown. The presence of the four functional groups was tested in the order of their explanatory power in the model. Two- and three-way interactions of functional group effects were small and were therefore omitted from the model. All error terms are printed in italics.

	df	**SS**	***F***	***P***
Spatial variation	9	42165	1.66	0.115
Species richnesss	1	38314	13.61	<0.001
Grass presence	1	4248	1.51	0.224
Small herb presence	1	3561	1.26	0.265
Legume presence	1	4541	1.61	0.209
Tall herb presence	1	0	0.00	1.000
Plot	*67*	*188639*	*1.58*	*0.027*
Seed addition	1	1522	0.85	0.359
Species richnesss × Seed addition	1	1535	0.86	0.357
Grass presence × Seed addition	1	31	0.02	0.896
Small herb presence × Seed addition	1	570	0.32	0.574
Legume presence × Seed addition	1	763	0.43	0.515
Tall herb presence × Seed addition	1	4455	2.49	0.118
Plot × Subplot	*76*	*135726*	*2.61*	*<0.001*
Year	1	5827	8.53	0.004
Species richnesss × Year	1	3751	5.49	0.020
Grass presence × Year	1	230	0.34	0.563
Small herb presence × Year	1	634	0.93	0.337
Legume presence × Year	1	39	0.06	0.811
Tall herb presence × Year	1	2636	3.86	0.051
Seed addition × Year	1	778	1.14	0.288
Plot × Subplot Year	*155*	*105942*	*0.31*	*1*
Residual	*160*	*353360*		

Table C5. Analysis of variance (ANOVA) of the total number of species per harvest quadrat and of community biomass (gm^{-2}). Target species were residents in weeded controls (w-), but residents, external and internal invaders in non-weeded subplots (c- and c+). "Species richnesss" is the sown species richnesss of the resident community. The weeding and seed-addition treatments (w-, c- and c+) were used to form two contrasts. The first of them ("Invasion") represents weeded controls (w-) vs. invasion treatments (c- and c+), the second ("Seed addition") represents non-weeded treatments without seed addition (c-) vs. with seed addition (c+). Error terms are printed in italics.

Source	Number of species				Biomass			
	df	SS	*F*	*P*	df	SS	*F*	*P*
Spatial variation	9	1100.7	9.05	<0.001	9	4440201	1.16	0.333
Species richnesss (log_2)	1	3655.3	270.36	<0.001	1	12139477	28.63	<0.001
Species richnesss (deviation from log-linear)	4	1206.8	22.32	<0.001	4	1651207	0.97	0.428
Plot	*67*	*905.8*	*3.28*	*<0.001*	*67*	*28408418*	*5.88*	*<0.001*
Invasion	1	2915.7	706.83	<0.001	1	5018898	69.59	<0.001
Seed addition	1	203.5	49.34	<0.001	1	251564	3.49	0.064
Species richnesss (log_2) × Invasion	1	289.2	70.10	<0.001	1	1080842	14.99	<0.001
Species richnesss (deviation from log-linear) × Invasion	4	0.8	0.05	0.995	4	445103	1.54	0.193
Species richnesss (log_2) × Seed addition	1	40.2	9.75	0.002	1	65241	0.90	0.343
Species richnesss (deviation from log-linear) × Seed addition	4	20.2	1.22	0.303	4	256648	0.89	0.472
Plot × Subplot	*150*	*618.8*	*0.99*	*0.522*	*150*	*10818315*	*0.75*	*0.970*
Year	1	663.5	159.32	<0.001	1	8354184	87.12	<0.001
Species richnesss (log_2) × Year	1	246.6	59.20	<0.001	1	38361	0.40	0.528
Species richnesss (deviation from log-linear) × Year	4	36.3	2.18	0.072	4	1745162	4.55	0.001
Invasion × Year	1	357.2	85.75	<0.001	1	1586364	16.54	<0.001
Seed addition × Year	1	114.4	27.46	<0.001	1	61793	0.64	0.423
Species richnesss (log_2) × Invasion × Year	1	87.4	20.98	<0.001	1	59669	0.62	0.431
Species richnesss (deviation from log-linear) × Invasion × Year	4	7.2	0.43	0.787	4	203245	0.53	0.714
Species richnesss (log_2) × Seed addition × Year	1	12.6	3.02	0.084	1	87566	0.91	0.340
Species richnesss (deviation from log-linear) × Seed addition × Year	4	5.2	0.31	0.868	4	291002	0.76	0.553
Plot × Subplot × Year	*224*	*932.9*	*2.13*	*<0.001*	*224*	*21480355*	*1.41*	*0.005*
Residual	*242*	*472.5*			*242*	*16501297*		

Table C6. Analysis of variance (ANOVA) of community biomass, including the effect of observed (=realized) species richnesss. Target species were residents in weeded controls (w-), but residents, external and internal invaders in non-weeded subplots (c- and c+). The influence of "realized species richnesss (log_2-transformed)" on community biomass was tested against the interaction "*Plot* × *Subplot* × *Year*". The weeding and seed-addition treatments (w-, c-, and c+) were used to form two contrasts. The first of them ("Invasion") represents weeded controls (w-) vs. non-weeded treatments (c- and c+), the second ("Seed addition") represents non-weeded treatments without seed addition (c-) vs. with seed addition (c+). Error terms are printed in italics.

Source	**df**	**SS**	***F***	***P***
Spatial variation	9	4085251	6.00	0.307
realized richness (log_2)	1	15619722	160.67	<0.001
Plot	*72*	*36869246*	*6.83*	*<0.001*
Invasion	1	75710	1.01	0.316
Seed addition	1	27015	0.36	0.549
realized richness (log_2) × Invasion	1	557075	5.73	0.017
realized richness (log_2) × Seed addition	1	658371	6.77	0.010
Plot × *Subplot*	*160*	*11987260*	*0.77*	*0.962*
Year	1	3874550	39.85	<0.001
realized richnesss (log_2) × Year	1	1850417	19.03	<0.001
Invasion × Year	1	1347	0.01	0.906
Seed addition × Year	1	15993	0.16	0.685
realized richness (log_2) × Invasion × Year	1	563858	5.80	0.017
realized richness (log_2) × Seed addition × Year	1	85679	0.88	0.349
Plot × *Subplot* × *Year*	*239*	*23234778*	*1.55*	*<0.001*
Residual	*233*	*14571877*		

Table C7. Analysis of variance (ANOVA) of the community biomass, including the effect of observed (=realized) functional-group proportions. In this analysis, external invaders were excluded because they could not be grouped into the respective functional groups, so target species in this case were residents in weeded controls (w-), but residents and internal invaders in non-weeded subplots (c- and c+). Three outliers with very high biomass were excluded. The realized functional-group proportions were included in the model in the order of their explanatory power and their influence was tested against the "*Residual*". The weeding and seed-addition treatments (w-, c-, and c+) were used to form two contrasts. The first of them ("Invasion") represents weeded controls (w-) vs. non-weeded treatments (c- and c+), the second ("Seed addition") represents non-weeded treatments without seed addition (c-) vs. with seed addition (c+). Error terms are printed in italics.

Source	**df**	**SS**	***F***	***P***
Spatial variation	9	2609852	2.10	0.041
Proportion of legumes	1	4847743	80.23	*<0.001*
Proportion of small herbs	1	1161391	19.22	*<0.001*
Proportion of grasses	1	1483	0.02	0.876
Plot	*72*	*9961269*	*2.29*	*<0.001*
Invasion	1	3875505	64.14	*<0.001*
Seed addition	1	142731	2.36	0.126
Proportion of legumes × Invasion	1	924491	15.30	*<0.001*
Proportion of small herbs × Invasion	1	41616	0.69	0.408
Proportion of grasses × Invasion	1	256598	4.25	0.041
Proportion of legumes × Seed addition	1	39499	0.65	0.420
Proportion of small herbs × Seed addition	1	9209	0.15	0.697
Proportion of grasses × Seed addition	1	410	0.01	0.934
Residual	*147*	*8882268*		

Chapter 8

What happens to the sown species if a biodiversity experiment is not weeded?

Roscher, C., Fergus, A.J.F., Petermann, J.A., Buchmann, N., Schmid, B., & Schulz, E-D. 2013. *Basic and Applied Ecology* 14: 187-198.

Abstract

Studies in experimental grasslands have extensively documented the effects of sown plant diversity on the colonization of new species, but the responses of the sown plant combinations themselves have rarely been investigated. We established experimental grasslands differing in species richness (1, 2, 4, 8, and 16) and functional group number and composition (1–4; legumes, grasses, small herbs, tall herbs), and we studied the changes in the abundance of sown species (residents) in both weeded and non-weeded subplots over a period of five years after sowing. The accumulation of new species through spontaneous colonization in the non-weeded treatment did not affect the number of resident species, but had increasingly negative effects over time on the cover of resident species and their aboveground biomass production at community level. Temporal stability of resident populations was lower and year-to-year changes in resident species composition were larger in non-weeded than in weeded subplots. Compositional dissimilarity between weeded and non-weeded treatments increased through time. These negative effects of the colonization of new species on the abundances and stability of resident populations depended on resident species identity and not on additional variation between different functional groups. The colonization of new species did not change the number of resident species emerging from seeds, but reduced seedling densities of residents. Colonization did not affect the structure of resident communities as measured by species evenness, functional trait diversity and mean trait values suggesting that colonization can destabilize the species composition of residents in terms of abundance while leaving them unchanged in terms of functional characteristics. Generally, negative impacts of colonizing species on residents which accelerated through time decreased with an increasing number of sown species. Sowing more diverse grassland mixtures increases their predictability in terms of ecosystem characteristics, which is important for ecological restoration and sustainable agriculture.

Introduction

Concerns about an accelerated loss of species diversity have stimulated an increasing interest in the potential impact of biodiversity on ecosystem processes (Hooper et al. 2005). Understanding the mechanisms that control community-level phenomena of assembly, compositional stability and resistance against invasion is

essential to assess consequences of species loss. More diverse plant communities are hypothesized to have a greater resistance against invasion (Elton 1958). Increased resource capture by a diverse community, leaving fewer resources available for potential invaders, has been suggested as an explanation (Tilman 1982). Invading species themselves may affect ecosystem processes by modifying species interactions and altering the structure and composition of the established communities (Meiners et al., 2001 and Yurkonis et al., 2005). Invaders may affect the resident community by inhibiting germination and establishment of new individuals belonging to the resident community (Crawley, Brown, Heard, & Edwards 1999). Invaders may also displace established individuals of resident species through resource competition or the development of antagonistic soil microbial feedbacks (Theoharides & Dukes 2007). The effects of invaders may depend on their identity and that of residents, however, effects at the species level may not necessarily translate into community processes (Yurkonis et al. 2005).

In spite of controversies over the interpretation of results about diversity–invasion resistance relationships obtained in observational and experimental studies (Fridley et al. 2007), similar mechanisms are supposed to explain the suppression of invaders of non-resident species by more diverse communities in natural and experimental systems. A number of biodiversity experiments have investigated the effects of species richness on the spontaneous colonization of new species at single or multiple points in time (e.g. Knops et al., 1999, van Ruijven et al., 2003 and Roscher et al., 2009a). Less is known about the response of the resident species themselves once a community is open to colonization by unsown species. Some studies reported a rapid loss of resident species in highly diverse mixtures in the first years after cessation of weeding (Pfisterer et al., 2004 and Rixen et al., 2008) or when high-diversity mixtures were established on arable land (Lepš et al. 2007). Such experiments did not include a weeded control and therefore lack the direct comparison of weeded vs. non-weeded artificially established communities. This distinction may have important implications for the evaluation of results obtained in numerous biodiversity experiments (Hooper et al. 2005), many of which have been criticized for immaturity of plant communities, their random species selection and the continuous manipulation required to maintain the designed species compositions (Wardle, 2001 and Lepš, 2004). In addition, land-use changes are among the most important drivers of global biodiversity and intensification of land-use is thought to

reduce the diversity and composition of biological communities (Schläpfer, Schmid, & Seidl 1999). Therefore, developing strategies to maintain, create or reassemble communities resistant to biological invasion is a major challenge for ecological restoration (Funk, Cleland, Suding, & Zavaleta 2008).

In an initial report of a study comparing a weeded and a non-weeded treatment in experimental grassland communities sown at different plant diversity levels (Jena Experiment; Roscher et al. 2004), we showed that the number and the abundance distribution of established resident species as well as their productivity were similar in both treatments in the first two years after sowing (Roscher, Temperton, Buchmann, & Schulze 2009). Here, we expand this study to look at the longer-term effects of colonizing species on the composition of resident species and their productivity over a 5-year time span. Based on the expectation that the impact of newly colonizing species accelerates through time in the non-weeded treatment by altering resource dynamics and species interactions we tested the following hypotheses: (1) Temporal stability in population- and community-level characteristics of residents is lower in non-weeded communities than in weeded communities. (2) Extinctions or reductions in the abundances of resident species are more pronounced in non-weeded communities. (3) Cover and productivity of residents are lower in non-weeded communities. (4) Shifts in species abundance distributions reduce evenness and functional trait diversity of residents in non-weeded communities because subordinate species are at a greater risk for displacement by newly colonizing species. (5) Differences in population and community characteristics of residents between non-weeded and weeded communities decrease with increasing richness of resident species.

Materials and Methods

Study site and experimental design

This study is part of a large biodiversity experiment (Jena Experiment; Roscher et al. 2004). The experimental site is situated in the floodplain of the river Saale at the northern edge of Jena (Jena-Löbstedt, Thuringia, Germany, 50°57′8″ N, 11°37′16″ E, 130 m a.s.l.). The area around Jena is characterized by a mean annual air temperature of 9.3 °C and mean annual precipitation of 587 mm (Kluge & Müller-Westermeier 2000). The soil of the experimental site is a Eutric Fluvisol developed from up to 2 m thick loamy fluvial sediments. Soil texture ranges from sandy loam near the river to silty clay with increasing distance from the river.

A pool of 60 species common in Molinio-Arrhenatheretea grasslands (semi-natural Central European mesophilic grasslands, Ellenberg 1988) was chosen for the experiment. These species were categorized into four functional groups: grasses (16 species), small herbs (12 species), tall herbs (20 species), and legumes (12 species). In total, the Jena Experiment comprises 78 plots of a size of 20 m × 20 m, which cover a gradient in species richness (1, 2, 4, 8, and 16) and functional group richness (1–4), the latter being near orthogonal to species richness. Mixtures were created by random draws with replacement. Each species-richness level was established on 16 large plots, except for the 16-species-richness level which was established on 14 large plots (because the species number in the legume and the small herb functional groups was too low for pure 16-species mixtures). In addition, two replicated monocultures of each experimental species were established on smaller plots of 3.5 m × 3.5 m. Plots were sown with a constant total density of 1000 germinable seeds per m2 distributed equally among species in mixtures. The experimental plots were arranged in four blocks parallel to the riverside to account for the gradient in soil characteristics.

Within each large plot, two subplots of 2.00 m × 2.25 m size separated by 0.3 m between one another were established near to the plot margin (excluding the outer 0.5 m buffer) shortly after the biodiversity experiment was sown in 2002. One of these subplots was weeded regularly as the main experiment (April, July). The other subplot was never weeded after sowing. Plots were mown twice each year (early June, September) and mown biomass was removed. The weeded control subplot was maintained until 2007.

Data collection

Aboveground biomass was harvested twice per year at estimated peak standing biomass (late May, August) just prior to mowing during the study period (2003–2007). The vegetation was clipped at 3 cm aboveground in a randomly located rectangle of 0.2 m × 0.5 m size in each subplot. Biomass was sorted into total resident (sown) species, total colonizer species and detached dead plant material. Samples were weighed after being dried to a constant weight (70 °C, 48 h). Total cover of resident and colonizer species was visually estimated to the nearest percentage before weeding (April, July) and again at estimated peak biomass (late May, August). Species cover was recorded twice per year directly before biomass harvest using a modified Londo scale (Londo 1976). Numerical values for species cover were coded as 0.5 (<1%), 3 (1–5%) 10 (6–15%), 20 (16–25%), 30 (26–35%), 40 (36–45%), 50 (46–55%), 60 (56–65%), 70 (66–75%), 80 (76–85%), and 90 (>85%). Seedlings (plant individuals with cotyledons) of resident species were counted three times in 2006 (April, July, October). Three quadrats (0.3 m × 0.3 m) per subplot were randomly placed for each census. Although it is not possible with this procedure to completely exclude the emergence of additional seedlings between these three time points (underestimation of seedling densities) or the persistence of seedlings for a longer period in this stage (overestimation of seedling densities), seedling densities per m2 were calculated for each subplot based on pooled data from all census points.

Aboveground plant traits were measured in monocultures of each species in May and August 2004. These traits were plant height, specific leaf area (SLA), leaf nitrogen concentration (NM) and shoot biomass:N ratios. Root characteristics (root depth, root type) were compiled as categorical variables from the literature (see Roscher et al. 2004 for details).

Data analyses

First, plant trait data and relative abundances of resident species were used to calculate community-weighted mean traits (CWMs; Garnier et al. 2004) and functional trait diversity (FD_Q) using Rao's quadratic diversity (Rao's Q; Rao 1982) for each subplot. Second, cover abundances of resident species were used to derive Shannon's evenness *J*, which is known to give greater weight to rare species, and Simpson's index of evenness $E_{1/D}$, which gives more weight to abundant species (

Smith & Wilson 1996). Third, cover values were used to compute Bray–Curtis distances (Bray & Curtis 1957) for resident species as a measure of compositional dissimilarity (1) between non-weeded vs. weeded subplot pairs per sampling date and (2) per subplot to assess year-to-year compositional changes. All calculations were completed separately for data recorded in early summer (May) and late summer (August) and averaged to obtain mean values for each year (for details see Appendix A).

To evaluate effects of non-weeding vs. weeding, the log response ratio

$$\ln RR_X = \ln\left(\frac{X_{\text{non-weeded}}}{X_{\text{weeded}}}\right)$$

Hedges, Gurevitch, and Curtis (1999) were computed between non-weeded vs. weeded subplots for all variables. Positive values of $\ln RR_X$ indicate that the studied variable increased in response to non-weeding, while negative $\ln RR_X$-values indicate a decrease in response to non-weeding. The coefficient of variation (CV) was calculated as a measure of temporal variability (McCann 2000) based on annual values of resident community characteristics and species abundances in weeded and non-weeded subplots (2003–2007).

Generalized linear models (type-I sum of squares) were used to test the effects of block, sown species richness (SR, log-linear term) and functional group number (FG, linear term) on variables measured at the plot level. In analyses of CVs, a split-plot term for non-weeded vs. weeded subplots, and in analyses of lnRR, a term for repeated measures and their interactions with SR and FG were entered. In a series of alternative models, contrasts for the presence of each functional group (legumes, grasses, small herbs, tall herbs) were fitted after SR and FG. To account for the unbalanced occurrences of sown species in the experimental plots, mixed effects models were applied in analyses of CVs and lnRR of individual species. Block and plot identity were entered as random effects in a nested sequence. Starting from a constant null model, terms for sown species richness (SR, log-linear), species identity, SR × species identity, weeding treatment and time, plus their interactions with SR and species identity were added sequentially as fixed factors. In alternative models, species identity was replaced by terms for functional group identity or

contrasts for each functional group. The maximum likelihood method was applied, and likelihood ratio tests (*L* ratio) were used to assess the statistical significance of model improvement. Data analysis was performed with the statistical software R2.11.1 (R Development Core Team, http://www.R-project.org), the implemented packages *nlme* (Pinheiro et al. 2009) and FD (Laliberté & Shipley 2010).

Results

Resident species richness

The temporal variability (measured as CV) of resident species richness increased with the number of sown species (Appendix A: Table S1, Fig. 1A). Log response ratios in resident species richness (lnRRSR) became increasingly negative through time, suggesting an increasing loss of resident species in the non-weeded subplots (Fig. 1B). Species loss was accelerated by legume presence in the sown species combinations (increasingly negative lnRRSR over time), while species loss was less pronounced in communities with small herbs. However, lnRRSR was not different from zero across the study period, indicating no significant differences in resident species richness between weeded and non-weeded subplots.

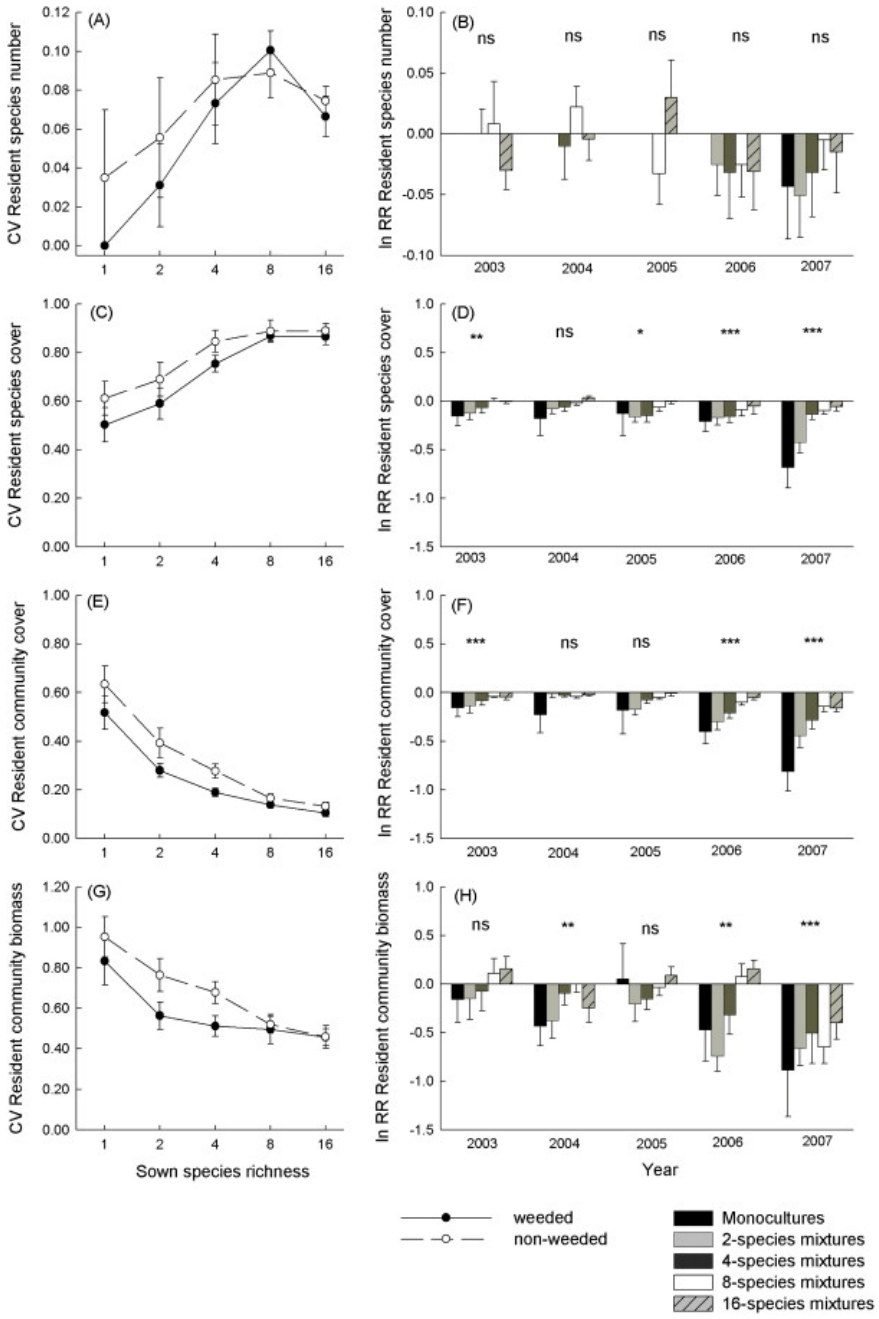

Fig. 1. Temporal variation (2003–2007) of resident species numbers (A, B), resident species cover (C, D), resident community cover (E, F), resident community biomass (G, H). In the left panels, means of CVs (±SE) are plotted against sown species richness for weeded and non-weeded subplots (A, C, E, G). In the right panels, lnRR-values are shown representing log response ratios between non-weeded and weeded subplots. They are given as means (±SE) for each sown species-richness level per study year (B, D, F, H). Positive lnRRs indicate that values were larger in non-weeded subplots compared to weeded subplots, while negative lnRRs indicate the opposite. Significance of overall means ≠ 0 across all species-richness levels was tested separately for each study year, where ns = non-significant, * $p \leq 0.050$, ** $p \leq 0.010$, *** $p \leq 0.001$.

Resident species abundance

The average temporal variability in resident species abundance increased with the number of sown species (Table S1, Fig. 1C). The temporal variability in resident species abundances was higher in non-weeded subplots, especially in communities with a lower number of sown species. Log response ratios of resident species abundances (lnRRPop) were significantly different from zero in all study years (with exception of 2004, Fig. 1D), indicating lower abundances of resident species in non-weeded compared to weeded subplots. Generally, differences in species abundances between weeded and non-weeded subplots decreased with a higher number of sown species (less negative $lnRR_{Pop}$). The deviation in species abundances in non-weeded from weeded subplots increased through time, particularly in communities with a lower number of sown species (more negative $lnRR_{Pop}$, Table S1).

Analyses at the species-level showed that the temporal variability in species abundances depended on species and functional group identity (Table 1). Effects of weeding vs. non-weeding on species temporal variability were not significantly different among species (Table 1). Differences in species abundances in response to weeding and their changes through time (i.e. $lnRR_{Pop}$ at species-level) were also dependent on species identity (Table 1). In total, only 5 out of 60 species had $lnRR_{Pop}$ significantly <0, i.e. lower abundances in non-weeded subplots, across all species-richness levels in the last study year (see Appendix A: Fig. S1). The $lnRR_{Pop}$ was highly variable, but mostly negative, for other species.

Table 2: Summary of mixed-effects model analyses for coefficients of variation for of resident species cover and log response ratios of resident species cover (lnRR comparing never-weeded vs. regularly-weeded subplots) based on a five-year study period from 2003–2007

	CV Species cover			ln RR Species cover	
	L ratio	*p*		*L* ratio	*p*
SR (log-linear)	**19.92**	**<0.001** ↓	Species number (SR)	**29.05**	**<0.001** ↑
Species ID	**806.33**	**<0.001**	Species ID	**89.86**	**0.006**
Functional group ID	**14.03**	**0.003**	Functional group ID	4.10	0.251
Legume	**6.25**	**0.012** ↑	Legume	0.25	0.617
Grass	0.29	0.208	Grass	2.10	0.147
Small herb	**11.8**	**0.001** ↓	Small herb	0.03	0.867
Tall herb	0.2	0.657	Tall herb	3.22	0.073
Weeding Treatment (W)	**4.51**	**0.034** ↑	Year	**22.44**	**<0.001**
SR x W	1.29	0.256	SR x Year	**9.04**	**0.003**
Species ID x W	19.69	1.000	Species ID x Year	**78.06**	**0.049**
Functional group ID x W	0.21	0.977	Functional group ID x Year	1.91	0.592
Legume x W	<0.01	0.955	Legume x Year	1.42	0.234
Grass x W	0.11	0.743	Grass x Year	0.06	0.801
Small herb x W	0.15	0.699	Small herb x Year	0.58	0.446
Tall herb x W	0.01	0.915	Tall herb x Year	0.44	0.509

Models were fitted by stepwise inclusion of fixed effects. Likelihood ratio tests were applied to assess model improvement (*L* ratio) and the statistical significance of the explanatory terms (*p* values). Significant effects are marked in bold. Arrows indicate increase (↑) or decrease (↓) of the variables with species richness or dependent on functional group identity.

Resident community cover and biomass production

Temporal variability in resident community cover and biomass production decreased with the number of sown species (Table S1, Fig. 1E and G). On average, temporal variability in resident community cover and biomass production was larger in non-weeded subplots, but differences between weeding treatments decreased with increasing numbers of sown species (Table S1).

Log response ratios closer to zero in resident community cover ($lnRR_{Cov}$) and biomass production ($lnRR_{Biom}$) indicated decreased differences between weeding treatments with increasing number of sown species (Table S1, Fig. 1F and H). On average, $lnRR_{Cov}$ and $lnRR_{Biom}$ decreased through time, but the decline in $lnRR_{Cov}$ was stronger when there were fewer sown species. From 2006 onwards, $lnRR_{Cov}$ and $lnRR_{Biom}$ were significantly lower than zero, indicating a lower community cover and biomass production in non-weeded compared to weeded subplots across all species-richness levels (Fig. 1F and H).

Number of germinating species and seedling density of resident species

The log response ratio of the number of germinating resident species ($lnRR_{SRgerm}$) based on three censuses in 2006 was not significantly different from zero (test for overall mean ≠ 0: $F_{1,72} = 0.33$, $p = 0.568$), suggesting that weeding treatments did not affect the number of germinating resident species (Fig. 2A) irrespective of sown species richness ($F_{1,72} = 0.90$, $p = 0.346$) or functional group number ($F_{1,72} = 0.05$, $p = 0.832$). The log response ratio of seedling densities of resident species ($lnRR_{Seed}$) was significantly lower than zero (test for overall mean ≠ 0: $F_{1,72} = 9.78$, $p = 0.003$), indicating that less resident seedlings emerged in the non-weeded subplots (Fig. 2B). The reduction of seedling emergence in non-weeded compared to weeded subplots did not depend on sown species richness ($F_{1,72} = 0.94$, $p = 0.336$) or functional group number ($F_{1,72} = 0.10$, $p = 0.758$).

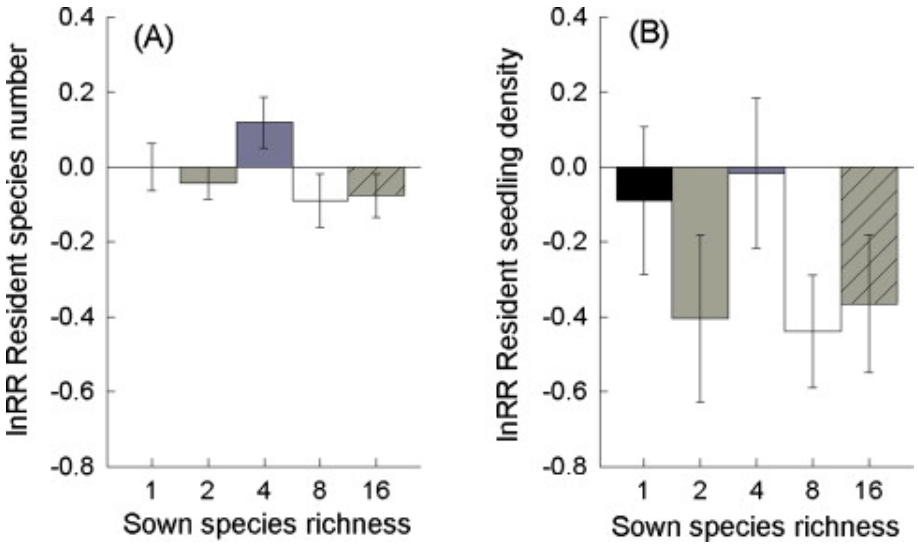

Fig. 2. The log response ratio (lnRR) of the number of germinating resident species (A), and the log response ratio of seedling densities of resident species (B) based on three censuses in 2006 (April, July, October) plotted against sown species richness. Bars represent means per species-richness level (±SE, for symbols see Fig. 1). Positive lnRRs indicate that numbers of germinating species and resident seedling densities were higher in non-weeded than in weeded subplots, while negative lnRRs indicate the opposite.

Compositional dissimilarity of resident species combinations

Compositional dissimilarity of resident species combinations (Bray–Curtis distances) between weeded and non-weeded subplots decreased with sown species richness ($F_{1,72} = 11.93$, $p = 0.001$), while functional group number or the presence of particular plant functional groups did not affect this compositional dissimilarity. Bray–Curtis distances between weeded and non-weeded treatments increased through time ($F_{1,309} = 64.58$, $p < 0.001$). This increase in dissimilarity was larger in species-poor compared to species-rich communities ($F_{1,309} = 23.61$, $p < 0.001$, Fig. 3).

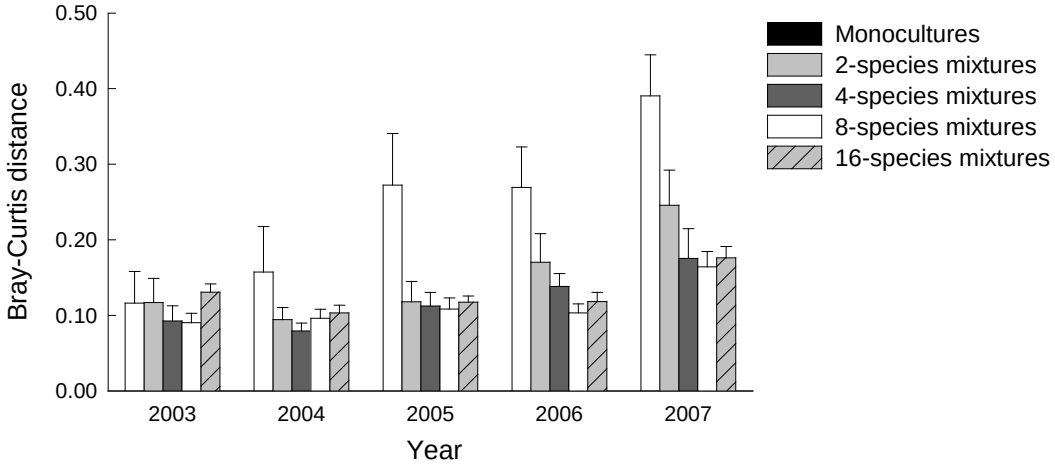

Fig. 3. Compositional dissimilarity (Bray–Curtis distances) of resident species combinations between pairs of weeded and non-weeded subplots by species-richness level (mean ± SE) and year. Values are based on two cover estimates (before the first (May) and second (August) mowing, respectively).

Temporal variability in resident species composition (CV of Bray–Curtis distances) decreased with increasing number of sown species, while functional group number, the presence of particular plant functional groups or weeding treatments did not affect temporal variability in composition (Appendix A: Table S2, Fig. 4A). Differences in temporal changes in terms of composition between weeded and non-weeded subplots decreased with increasing number of sown species (decreasing $\ln RR_{Comp}$, Table S2), but they became stronger through time (Table S2). The $\ln RR_{Comp}$ from 2006 to 2007 was significantly larger than zero, suggesting that temporal changes in non-weeded subplots exceeded those in weeded subplots across all species-richness levels (Fig. 4B).

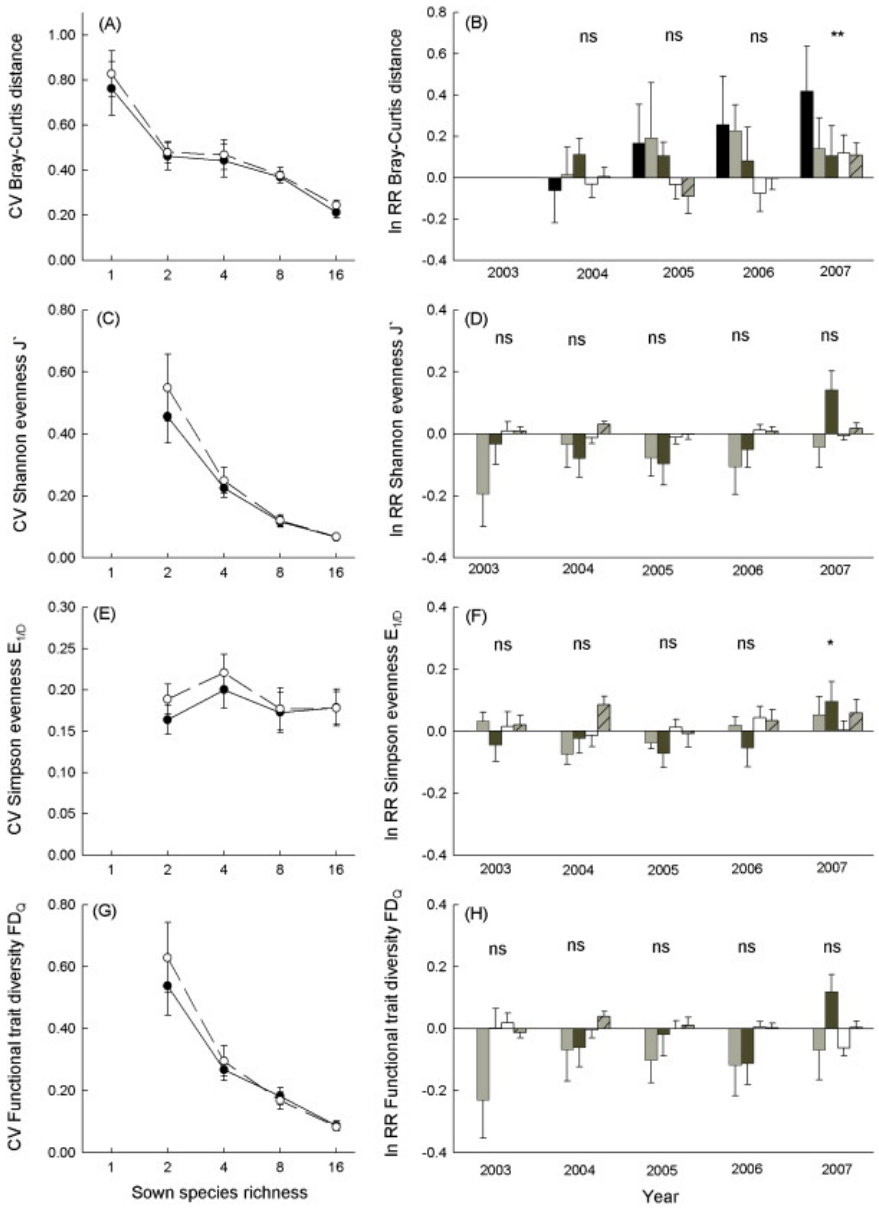

Fig. 4. Temporal variation (2003–2007) of year-to-year changes (Bray–Curtis distances) in resident species composition (A, B), Shannon evenness *J'* of resident species combinations (C, D), Simpson index of evenness $E_{1/D}$ of resident species combinations (E, F), functional trait diversity FD_Q (G, H). In the left panels, means of CVs (±SE) are plotted against sown species richness for weeded and non-weeded subplots (A, C, E, G). In the right panels, lnRR-values are shown representing log response ratios between non-weeded and weeded subplots. They are given as means (±SE) for each sown species-richness level per study year (B, D, F, H). Positive lnRRs indicate that values were larger in non-weeded subplots compared to weeded subplots, while negative lnRRs indicate the opposite. Significance of overall means ≠ 0 across all species-richness levels was tested separately for each study year, where ns = non-significant, * $p \leq 0.050$, ** $p \leq 0.010$, *** $p \leq 0.001$. For symbols see Fig. 1.

Evenness of resident species combinations

The temporal variability of the Shannon evenness (J') decreased with increasing sown species richness, while the CV of the Simpson index of evenness ($E_{1/D}$) was not influenced by species richness, indicating that species-rich communities had a more stable species abundance distribution of subordinate species (Table S2, Fig. 4C and D). The temporal variability of J' and $E_{1/D}$ did not differ between weeded and non-weeded subplots. Differences in J' between weeding treatments decreased at higher sown species-richness levels (increasing $\ln RR_{Shan}$, Table S2, Fig. 4D). In general, weeded and non-weeded subplots did not differ significantly in species evenness in the 5-year study (Fig. 4D and F), except for a higher $E_{1/D}$ across all species-richness levels in the non-weeded subplots in 2007 ($\ln RR_{Simp} > 0$; Fig. 4F).

Functional trait diversity and aggregated traits of resident species combinations

Temporal variability in single-trait as well as in multiple-trait functional diversity FD_Q decreased with an increasing number of sown species or functional groups, with the exception of FD_Q in root depth (Appendix A: Table S3, Fig. 4G). Temporal variability in community-weighted means of trait values (CWM) did not depend on sown species richness, but increasing functional group richness was coupled with increased temporal variability in community-weighted leaf nitrogen concentrations (N_M) and shoot biomass:N ratios (Table S3). Temporal variability in functional trait composition (CWM and FD_Q) was not influenced by weeding (Table S3). Log response ratios ($\ln RR_{CWM}$, $\ln RR_{FDQ}$) were not significantly different from zero (test for overall mean ≠ 0: $p > 0.05$), indicating that functional trait composition of residents between weeded and non-weeded subplots across all species-richness levels did not differ (Fig. 4H). However, $\ln RR_{FDQ}$ in multiple traits, but also $\ln RR_{FDQ}$ in single traits such as N_M and shoot height, became increasingly negative at lower sown species richness, suggesting that non-weeding had negative effects on FD_Q (Table S3, Fig. 4H).

Cover and species number of colonizers

On average, colonizer cover and the number of colonizing species decreased with increasing sown species richness (Appendix A: Table S4, Fig. 5). Weeding reduced colonizer cover and colonizer species number. Effects of weeding on colonizer cover

did not depend on sown species richness, while colonizer species numbers were more successfully reduced through weeding at increasing species richness (Table S4). Overall, colonizer cover and species numbers increased with increasing time after sowing of the biodiversity experiment. Weeding did not prevent the establishment of a higher number of colonizer species through time, while the increase in colonizer cover through time was less pronounced when communities were weeded (Table S4).

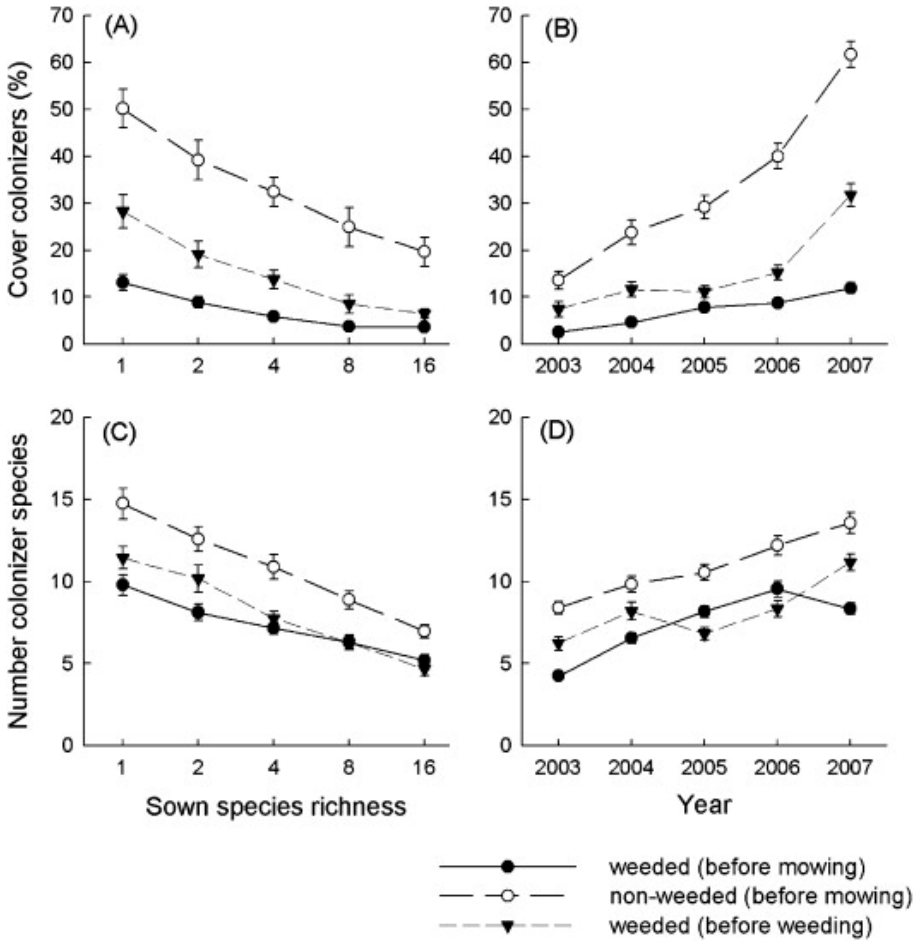

Fig. 5. Cover of colonizer species plotted against sown species richness (A) and year (B), and colonizer species numbers plotted against sown species richness (C) and year (D) in weeded subplots before weeding (mean values for data recorded before spring- and summer-weeding) and mowing (mean values for data recorded before early- and late-summer mowing) and non-weeded subplots before mowing. Values are means (±SE) per species-richness level across study years (2003–2007) in the left panels, and means (±SE) across all species-richness levels per study year in the right panels.

Discussion

The goal of the present study was to test what would happen to the sown species over several years if a biodiversity experiment was not weeded. Typically, most plant biodiversity experiments weed unwanted species in order to maintain sown species-richness levels and community composition (for an exception, see Niklaus, Leadley, Schmid, & Körner 2001). This is justified as the simulated extinction of all other species from a community (Schmid & Hector 2004). However, weeding may be seen as an undesirable disturbance or unusual management practice (Wardle, 2001 and Lepš, 2004) generating doubt about whether similar results would be observed if biodiversity experiments were not weeded. Our study comparing weeded and non-weeded subplots in a grassland biodiversity experiment showed that newly colonizing species may reduce the temporal stability of resident population- and community-level characteristics (confirming hypothesis 1). While species and community cover as well as productivity were reduced in non-weeded communities (confirming hypotheses 2 and 3), the shift in abundance distribution had minor effects on community characteristics such as evenness and functional trait composition (rejecting hypothesis 4). In general, impacts of colonization by new species were moderate in communities with higher species richness, while their negative effects became more severe in communities with lower species richness over several years (confirming hypothesis 5).

Negative effects of newly colonizing species on community structure and composition of resident species may involve direct competitive displacement as well as inhibition of establishment of new individuals. The competition–colonization model (Tilman 1994) is based on the assumption that resident species are excluded and the local diversity is reduced when competitive, dominant species are introduced. In our study, resident species numbers in weeded and non-weeded subplots were not different, but tended to diverge over the 5-year study period. However, a higher sown diversity increased the temporal variability of resident species numbers irrespective of weeding (Fig. 1A). This suggests that observations of increased species extinctions at higher species richness after cessation of weeding in studies lacking a continuously weeded control (Pfisterer et al., 2004 and Rixen et al., 2008) may not necessarily have been caused by the colonization of new species but by a reduced compositional stability of communities with a higher number of less stable subordinate species (Foster et al., 2002 and Roscher et al., 2011). In addition, the

pressure of colonizers might have been larger in previous experiments with smaller plots (Pfisterer et al., 2004 and Rixen et al., 2008), while the non-weeded subplots in the large plots of Jena Experiment were surrounded by a weeded area with the same sown species combinations.

In contrast to negligible effects of weeding treatments on the numbers of resident species, average resident species cover was lower in non-weeded communities and compositional divergence between weeding treatments increased through time. As a consequence, non-weeded communities had on average a lower stability in biomass production and total plant cover of residents compared to weeded communities (Fig. 1E and G). However, temporal stability in productivity and cover of residents was positively related to sown plant diversity irrespective of weeding, which is in line with a previous study by Bezemer and van der Putten (2007) on ex-arable land.

One possible explanation for the reduction of resident species abundances and community biomass is resource competition between residents and colonizers. The availability of light and soil resources has been shown to regulate the success of colonizing species in several experimental studies in grasslands (e.g. Davis et al., 2000 and Roscher et al., 2009a). Grime (2006) predicted that traits associated with competition are more common in relatively undisturbed, productive environments, because species with traits associated with poor competitive ability are likely to be competitively excluded. Under nutrient-rich conditions fast growth is a prerequisite for high competitive ability. Specific leaf area and leaf nitrogen concentrations correlate positively with relative growth rates (e.g. van der Werf, van Nuenen, Visser, & Lambers 1993). In addition, plant height is an important indicator for species competitive ability (Gaudet & Keddy 1988). In our study, the spontaneous colonization of new species had only minor impacts on trait composition of dominant species as shown by the non-significant differences in community-weighted means of traits related to competitive ability between weeded and non-weeded communities. Only at lower levels of sown plant diversity did colonizing species have negative impacts on the diversity of light- (plant height) and nitrogen-acquisition strategies of the resident species (more negative values of lnRRFDQ, Fig. 4H, Table S3), suggesting that the accumulation of more colonizers reduced niche diversification among residents.

Negative soil feedbacks, i.e. the accumulation of pathogens, parasites or herbivores of roots (Bever, Westover, & Antonovics 1997), provide an alternative explanation for a declining performance of residents in our long-term experiment. Relative species abundances in plant communities may decline through reduced competitive ability when species are growing on the same soil for an extended time (Klironomos, 2002 and Petermann et al., 2008). The composition of soil organisms is likely to differ between non-weeded and weeded communities, where the removal of unwanted species causes soil disturbances and therefore could reduce the potential for negative soil feedbacks. A lower competitive ability of residents through antagonistic interactions with soil organisms would also favour the competitive displacement of residents through the accumulation of new colonizers in non-weeded communities. In addition, negative soil feedbacks could explain why reductions in the abundance of residents in non-weeded communities were common across nearly all experimental species and not restricted to subordinate species with lower ability for resource competition (Fig. S1).

Reduced establishment of new individuals of residents could further increase negative effects of colonizers in non-weeded communities in the long term. Higher seedling numbers of resident species in weeded communities (Fig. 2B) could be attributable to either a close relationship between reduced population sizes and propagule accumulation in non-weeded communities, a stimulation of germination caused by soil disturbance during weeding (Leck, Parker, & Simpson 1989), or the limitation of favourable microsites for germination in non-weeded plant communities, which had a higher total plant cover (analysis not shown).

The colonization of new species apparently prevented single species from attaining extensive dominance. In the long term, the Simpson index of evenness, which gives greater weight to abundant species, was higher in non-weeded than in weeded communities (Fig. 4F). In contrast, at lower species richness, the Shannon evenness, which accords rare species greater weight, was lower in non-weeded communities (Fig. 4D). Therefore, those resident species that occurred in low abundances were acutely impeded by the colonization of new species in communities of lower sown species richness. This consolidates the decreasing functional trait diversity in non-weeded communities in our study and the non-random species extinction scenarios in natural ecosystems (Zavaleta & Hulvey 2004). Several removal experiments in natural grasslands and abandoned agricultural land

have shown that the effects of removal of single species or functional groups on compensatory growth, the colonization of new species and subsequent community structure is dependent on the identity of the removed and the remaining species (e.g. Munson and Lauenroth, 2009 and McLaren and Turkington, 2011).

In summary, the observed changes in the abundance distribution of resident species and the compositional divergence between weeded and non-weeded communities suggest that the impact of colonizing species on resident species accelerates through time, particularly in communities with fewer initially sown species. It is well known from several studies in weeded experimental grasslands that their temporal stability increases with species richness (e.g. Tilman et al., 2006 and Roscher et al., 2011). Our study adds information showing that the patterns observed in weeded communities only apply at higher sown diversity when colonizing species are not removed through weeding. This is important for the evaluation of results obtained in biodiversity experiments and their implications for restoration and sustainable agriculture. Sowing more diverse grassland mixtures increases their predictability in terms of ecosystem characteristics such as productivity as well as their species and functional composition, which is critically important for interactions with organisms at higher trophic levels, which are usually more dependent on species composition than on intrinsic richness.

Acknowledgements

The Jena Experiment is funded by the German Science Foundation (FOR 456) with additional support from the Max Planck Society and the Friedrich Schiller University of Jena and coordinated by W.W. Weisser. B.S. was additionally supported by the Swiss National Science Foundation (310030E-131193). We thank V.M. Temperton for the provision of biomass data. We acknowledge U. Wehmeier, S. Junghans, S. Hengelhaupt, A. Oswald, U. Gerighausen and many student helpers for their support with the maintenance of the invasion experiment, harvest of biomass, measurement of plant traits and sample preparation for chemical analysis. I. Hilke carried out elemental analyses.

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Appendix A: Supplementary Material

Calculation of community-weighted mean traits (CWM)

$$CWM = \sum_{i=1}^{S} p_i t_i$$

where S is the number of resident species in the subplot, t_i are species-specific trait values, and p_i are abundance proportions of resident species in each subplot

Calculation of functional trait diversity (FD_Q) for single and multiple traits

$$FD_Q = \sum_{i=1}^{S} \sum_{j=1}^{S} p_i p_j d_{ij}$$

where d_{ij} is the trait distance between the i-th and j-th species for single or multiple traits respectively, p_i and p_j are the relative abundances of resident species i and j, and S is the number of resident species in the subplot

Calculation of measures of evenness

Shannon evenness $J` = \dfrac{-\sum_{i=1}^{S} (p_i)(\ln p_i)}{\ln S}$

Simpson index of evenness $E_{1/D} = \dfrac{1/\sum_{i=1}^{S} p_i^{\,2}}{S}$

where p_i is the relative abundance of species i, and S is the number of resident species in the subplot

Calculation of Bray-Curtis distances

$$BC = \frac{\sum_{i=1}^{S} | p_{ik} - p_{il} |}{\sum_{i=1}^{S} (p_{ik} + p_{il})}$$

where p_i is the relative abundance of species i in subplots k and l, and S is the number of resident species in the subplot

Fig. S1. Effects of non-weeding on resident (= sown) species cover as log response ratio (= lnRR) five years after establishment (2007) comparing non-weeded vs. weeded subplots. Bars represent means (± SE) across all communities where a particular species belonged to the resident species combinations. Positive lnRRs indicate that average cover of a species was higher in non-weeded subplots relative to weeded subplots, while negative lnRRs indicate the opposite. Significance of overall means ≠ 0 across all species-richness levels was tested separately for each species, where * $p \leq 0.050$, ** $p \leq 0.010$.

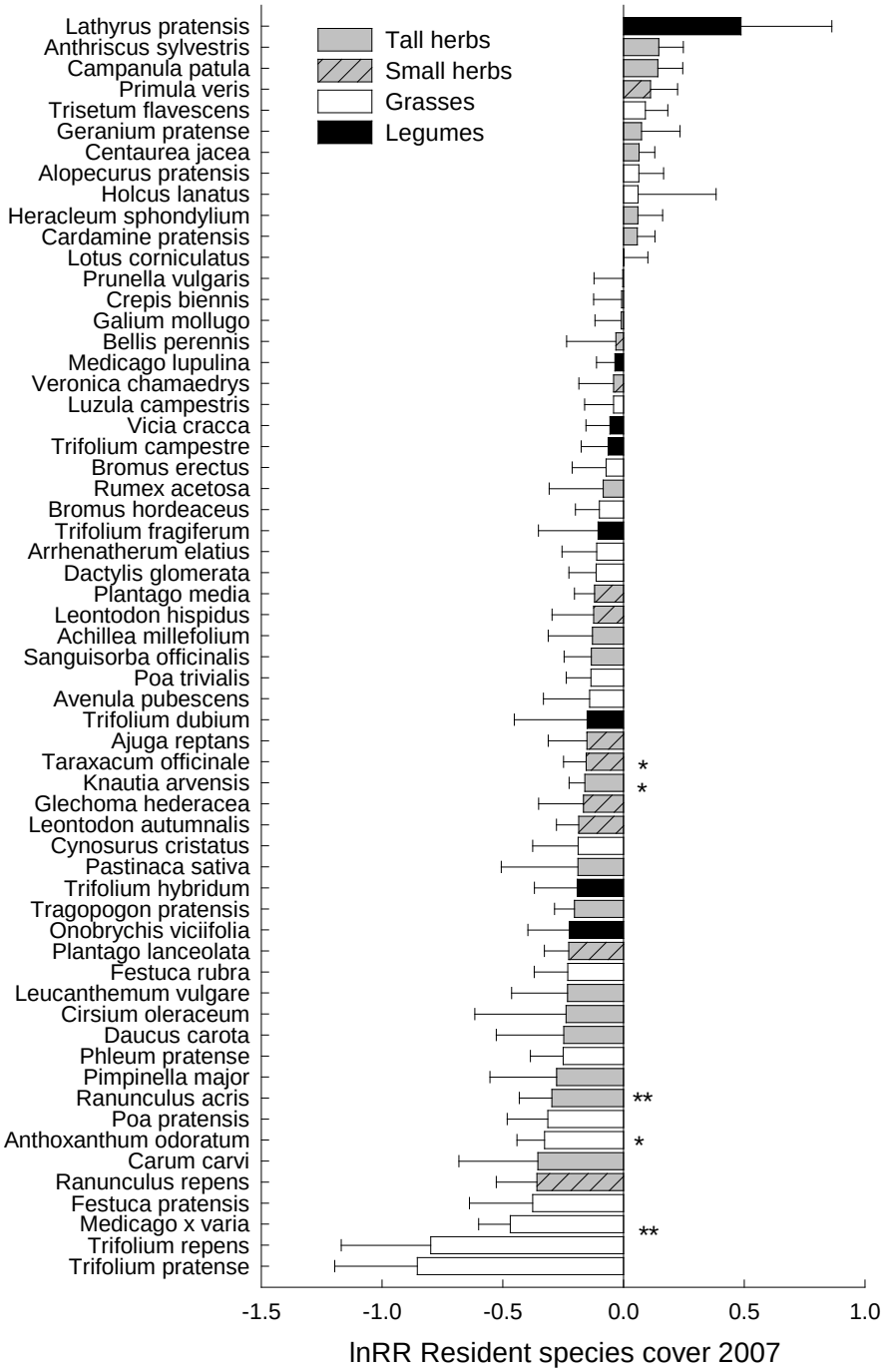

Table S1. Summary of generalized linear models (ANOVA) for coefficients of variation (CV) and log response ratios (lnRR comparing non-weeded vs. weeded subplots) for resident species numbers, average species cover, community cover and community biomass based on a five-year study period (2003–2007)

Source of variation	Species number				Species cover			Community cover			Community biomass		
	df	MS	F	p	MS	F	p	MS	F	p	MS	F	p
Coefficient of variation (CV)													
Block	3	0.002	1.16	0.332	0.024	0.32	0.810	0.006	1.50	0.221	0.104	0.81	0.492
SR (log-linear)	1	0.016	**10.62**	**0.002** ↑	1.981	**26.15**	**<0.001** ↑	0.370	**88.68**	**<0.001** ↓	3.235	**25.15**	**<0.001** ↓
FG (linear)	1	<0.001	0.04	0.851	0.003	0.03	0.855	0.007	1.60	0.211	0.163	1.27	0.264
Legume (LE)	1	0.024	**20.54**	**<0.001** ↑	0.198	2.68	0.106	<0.001	0.04	0.849	0.564	**4.60**	**0.035** ↓
Grass (GR)	1	0.008	**6.00**	**0.017** ↓	0.394	**5.53**	**0.022** ↓	0.007	1.69	0.197	0.061	0.47	0.497
Small herb (SH)	1	0.009	**6.53**	**0.013** ↓	0.017	0.22	0.643	0.001	0.22	0.638	0.159	1.24	0.269
Tall herb (TH)	1	0.001	0.47	0.495	0.085	1.13	0.292	0.002	0.36	0.549	0.015	0.12	0.733
Plot	72	0.001			0.076			0.004			0.129		
Weeding Treatment (W)	1	0.001	2.54	0.115	0.154	**19.90**	**<0.001**	0.021	**32.85**	**<0.001**	0.432	**11.19**	**0.001**
W x SR (log-linear)	1	0.001	2.02	0.159	0.057	**7.42**	**0.008**	0.003	**4.95**	**0.029**	0.125	3.25	0.076
W x FG (linear)	1	<0.001	0.04	0.843	<0.001	<0.01	0.964	<0.001	0.13	0.718	0.004	0.12	0.735
W x LE	1	0.003	**9.70**	**0.003**	0.033	**4.54**	**0.037**	0.001	1.05	0.308	0.025	0.64	0.426
W x GR	1	<0.001	1.28	0.261	<0.001	<0.01	0.987	0.001	1.09	0.301	0.037	0.95	0.332
W x SH	1	0.001	1.98	0.163	0.025	3.40	0.069	0.002	2.47	0.120	0.032	0.84	0.363
W x TH	1	<0.001	0.17	0.685	0.001	0.09	0.761	0.001	2.31	0.133	0.018	0.46	0.499
Residuals	75	<0.001			0.008			0.001			0.039		
log response ratio (lnRR)													
Block	3	0.026	1.67	0.181	0.741	3.35	0.024	0.288	1.11	0.352	0.282	0.37	0.775
SR (log-linear)	1	0.001	0.03	0.853	3.267	**14.76**	**<0.001** ↓	4.219	**16.20**	**<0.001** ↑	7.519	**9.86**	**0.002** ↑
FG (linear)	1	0.001	0.06	0.808	0.014	0.06	0.803	0.007	0.03	0.869	0.370	0.49	0.488
Legume (LE)	1	0.101	**7.21**	**0.009** ↓	0.296	1.34	0.250	0.357	1.38	0.244	3.108	**4.26**	**0.043** ↓
Grass (GR)	1	0.023	1.53	0.220	0.061	0.27	0.604	0.058	0.22	0.639	0.676	0.88	0.350
Small herb (SH)	1	0.021	1.37	0.245	0.764	3.58	0.063	1.172	**4.74**	**0.033** ↑	0.271	0.35	0.555
Tall herb (TH)	1	0.001	0.06	0.814	0.005	0.02	0.883	0.051	0.19	0.662	0.211	0.27	0.603
Plot	72	0.015			0.221			0.260			0.762		
Year	1	0.045	**6.22**	**0.013** ↓	1.968	**17.36**	**<0.001** ↓	3.951	**35.25**	**<0.001** ↓	11.905	**16.99**	**<0.001** ↓
Year x SR	1	0.008	1.07	0.303	0.781	**6.89**	**0.009**	1.504	**13.41**	**<0.001**	0.316	0.45	0.503
Year x FG	1	0.014	2.01	0.158	0.058	0.51	0.474	0.092	0.82	0.365	0.169	0.24	0.624
Year x LE	1	0.068	**9.64**	**0.002**	0.056	0.50	0.482	0.160	1.43	0.232	0.102	0.15	0.703
Year x GR	1	0.019	2.62	0.107	<0.001	<0.01	0.956	0.015	0.14	0.712	0.202	0.29	0.593
Year x SH	1	0.056	**8.00**	**0.005**	0.067	0.59	0.442	0.004	0.03	0.855	0.016	0.02	0.881
Year x TH	1	0.012	1.64	0.202	<0.001	<0.01	0.996	0.043	0.38	0.537	0.755	1.08	0.300
Residuals	309	0.007			0.113			0.112			0.701		

Model terms were fitted sequentially and tested against the respective residuals. Note that functional group identities were fitted as separate contrasts in series of analyses. Given are the degrees of freedom (df), mean sums of squares (MS), F ratios (F) and p values (p); significant effects are marked in bold. Arrows indicate increase (↑) or decrease (↓) of the variables with species richness, functional group number, presence of a particular functional group or time; SR = sown species richness, FG = functional group number.

Table S2. Summary of generalized linear models (ANOVA) for coefficients of variation (CV) and log response ratios (lnRR comparing non-weeded vs. weeded subplots) for year-to-year compositional dissimilarity (Bray-Curtis distances), Shannon evenness J` and Simpson index of evenness $E_{1/D}$ of resident species combinations based on a five-year study period (2003–2007)

Source of variation	Bray-Curtis distances				Shannon evenness J`				Simpson evenness $E_{1/D}$			
	df	MS	F	p	df	MS	F	p	df	MS	F	p
Coefficient of variation (CV)												
Block	3	0.754	1.96	0.127	3	1.792	2.71	0.053	3	0.016	1.42	0.246
SR (log-linear)	1	19.511	**50.84**	**0.001** ↓	1	56.991	**86.25**	**<0.001** ↓	1	0.001	0.06	0.808
FG (linear)	1	0.001	<0.01	0.953	1	0.990	1.50	0.226	1	0.033	2.92	0.093
Legume (LE)	1	0.514	1.34	0.250	1	0.066	0.10	0.754	1	0.029	2.61	0.112
Grass (GR)	1	0.996	2.65	0.108	1	1.571	2.44	0.124	1	<0.001	0.02	0.887
Small herb (SH)	1	0.266	0.69	0.409	1	1.500	2.32	0.133	1	0.003	0.30	0.589
Tall herb (TH)	1	0.577	1.51	0.223	1	4.657	**7.92**	**0.007** ↑	1	0.047	**4.36**	**0.041** ↑
Plot	72	0.384			56	0.661			56	0.011		
Weeding Treatment (W)	1	0.425	2.47	0.120	1	0.130	2.25	0.139	1	0.005	1.74	0.192
W x SR (log-linear)	1	0.004	0.03	0.873	1	0.075	1.29	0.260	1	0.003	1.15	0.288
W x FG (linear)	1	0.068	0.40	0.531	1	0.006	0.10	0.757	1	0.001	0.45	0.506
W x LE	1	0.021	0.12	0.728	1	0.002	0.03	0.861	1	<0.001	0.05	0.832
W x GR	1	0.042	0.24	0.623	1	0.039	0.67	0.418	1	0.005	1.70	0.197
W x SH	1	0.212	1.23	0.270	1	0.042	0.73	0.398	1	0.005	1.64	0.205
W x TH	1	0.009	0.05	0.825	1	0.002	0.03	0.871	1	<0.001	0.01	0.903
Residuals	75	0.172			59	0.058			59	0.003		
log response ratio (ln RR)												
Block	3	0.035	0.10	0.962	3	0.023	0.41	0.749	3	0.083	2.21	0.097
SR (log-linear)	1	1.724	**4.75**	**0.033** ↓	1	0.436	**7.57**	**0.008** ↑	1	0.073	1.94	0.169
FG (linear)	1	0.139	0.38	0.538	1	0.005	0.08	0.775	1	0.006	0.15	0.702
Legume (LE)	1	0.469	1.30	0.258	1	0.027	0.46	0.499	1	0.020	0.53	0.468
Grass (GR)	1	0.006	0.02	0.900	1	0.052	0.90	0.347	1	0.006	0.16	0.688
Small herb (SH)	1	0.528	1.47	0.230	1	0.004	0.07	0.787	1	0.005	0.13	0.717
Tall herb (TH)	1	0.010	0.03	0.871	1	<0.001	0.00	0.960	1	0.023	0.61	0.440
Plot	72	0.363			56	0.058			56	0.038		
Year	1	1.167	**4.06**	**0.045** ↑	1	0.147	3.34	0.069	1	0.079	3.10	0.080
Year x SR	1	0.422	1.47	0.227	1	0.091	2.07	0.152	1	0.023	0.90	0.343
Year x FG	1	0.495	1.72	0.191	1	0.005	0.12	0.733	1	0.008	0.31	0.576
Year x LE	1	0.099	0.34	0.559	1	0.258	**5.99**	**0.015**	1	0.107	**4.27**	**0.040**
Year x GR	1	0.052	0.18	0.672	1	0.244	**5.67**	**0.018**	1	0.029	1.16	0.283
Year x SH	1	0.531	1.85	0.175	1	0.106	2.43	0.120	1	0.036	1.43	0.232
Year x TH	1	0.372	1.30	0.256	1	0.093	2.13	0.146	1	0.001	0.04	0.837
Residuals	231	0.287			245	0.044			245	0.025		

Model terms were fitted sequentially and tested against the respective residuals. Note that functional group identities were fitted as separate contrasts in series of analyses. Given are the degrees of freedom (df), mean sums of squares (MS), F ratios (F) and p values (p); significant effects are marked in bold. Arrows indicate increase (↑) or decrease (↓) of the variables with species richness, functional group number, presence of a particular functional group or time; SR = sown species richness, FG = functional group number.

Table S3. Summary of generalized linear models (ANOVA) for coefficients of variation (CV) and log response ratios (lnRR comparing non-weeded vs. weeded subplots) for community-weighted mean traits (CWM) and functional trait diversity (FD_Q) of resident species based on a five-year study period (2003–2007)

	CWM N_M	CWM SLA	CWM Biomass:N ratio	CWM Height	CWM Root depth	CWM Root type	FD_Q N_M	FD_Q SLA	FD_Q Biomass:N ratio	FD_Q Height	FD_Q Root depth	FD_Q Root type	Mutiple-trait FD_Q
Coefficient of variation (CV)													
Block	-	-	-	-	-	-	-	-	-	-	-	-	-
SR (log-linear)	-	-	-	-	-	-	***↓	***↓	***↓	***↓	-	-	***↓
FG (linear)	***↑	-	***↑	-	-	-	-	-	-	-	-	*↓	*↓
Legume (LE)	-	-	*↑	-	*↓	*↓	-	-	-	-	-	-	-
Grass (GR)	-	*↑	**↓	-	-	-	-	-	-	-	-	*↓	-
Small herb (SH)	-	-	-	*↑	**↑	*↑	-	-	-	-	-	-	*↓
Tall herb (TH)	-	-	-	-	-	-	-	-	-	-	-	*↑	**↑
Weeding Treatment (W)	-	-	-	-	-	-	-	-	-	-	-	-	-
W x SR (log-linear)	-	*	-	-	-	-	-	-	-	-	-	-	-
W x FG (linear)	-	-	-	-	-	-	-	-	-	-	-	-	-
W x LE	-	-	-	-	-	-	*	-	-	-	-	-	-
W x GR	-	-	-	-	-	-	-	-	-	-	-	-	-
W x SH	-	-	-	-	-	-	-	-	-	-	-	-	-
W x TH	-	-	-	-	-	-	-	-	-	-	-	-	-
log response ratio (ln RR)													
Block	-	-	-	-	-	-	-	-	-	-	-	-	-
SR (log-linear)	-	-	-	-	-	-	*↑	-	-	*↑	-	-	*↑
FG (linear)	-	-	-	-	-	-	-	-	-	-	-	-	-
Legume (LE)	-	-	-	*↑	-	*↑	-	-	-	-	-	-	-
Grass (GR)	-	*↓	-	*↓	-	-	-	-	-	-	-	-	-
Small herb (SH)	-	-	-	-	-	-	-	-	-	-	-	-	-
Tall herb (TH)	-	-	-	-	-	-	-	-	-	-	-	-	-
Year	-	-	-	-	-	-	-	*↑	-	-	-	-	-
Year x SR	-	-	-	-	-	-	-	-	-	-	-	-	-
Year x FG	-	-	-	-	-	-	-	-	-	-	-	-	-
Year x LE	-	-	-	-	-	-	-	-	-	-	-	-	-
Year x GR	-	-	-	-	-	-	-	**	-	*	-	-	*
Year x SH	-	*	-	-	-	-	*	*	-	-	-	-	*
Year x TH	-	-	-	-	-	-	*	-	-	-	-	-	-

Model terms were fitted sequentially and tested against the respective residuals. Note that functional group identities were fitted as separate contrasts in series of analyses. Listed are the results of the statistical significance of the variables, where *: $p \leq 0.050$, **: $p < 0.010$, and ***: $p < 0.001$. Arrows indicate increase (↑) or decrease (↓) of the variables with species richness, functional group number, presence of a particular functional group or time; SR = sown species richness, FG = functional group number, SLA = specific leaf area, N_M = leaf nitrogen concentration.

Table S4. Summary of generalized linear models (ANOVA) for colonizer cover and colonizer species number at estimated peak biomass before mowing (means for early and late summer) based on a five-year study period (2003–2007) in weeded and non-weeded subplots

Source of variation	Colonizer cover					Colonizer species number			
	df	MS	F	p		MS	F	p	
Block	3	0.63	1.70	0.176		38.70	0.85	0.469	
SR (log-linear)	1	17.43	**46.93**	**<0.001**	↓	3459.80	**76.43**	**<0.001**	↓
FG (linear)	1	0.03	0.07	0.796		122.20	2.70	0.105	
Legume (LE)	1	4.39	**13.92**	**<0.001**	↑	276.60	**6.59**	**0.012**	↑
Grass (GR)	1	3.95	**12.30**	**0.001**	↓	22.30	0.49	0.487	
Small herb (SH)	1	1.40	3.94	0.051		98.40	2.21	0.142	
Tall herb (TH)	1	1.00	2.75	0.102		134.60	3.06	0.085	
Plot	72	0.37	**4.13**	**<0.001**		45.30	**5.12**	**<0.001**	
Weeding treatment (W)	1	67.51	**746.92**	**<0.001**		2406.28	**272.16**	**<0.001**	
W x SR (log-linear)	1	0.16	1.75	0.190		262.09	**29.64**	**<0.001**	
W x FG (linear)	1	0.00	0.02	0.881		4.13	0.47	0.497	
W x Legume (LE)	1	0.42	**4.83**	**0.031**		13.18	1.50	0.225	
W x Grass (GR)	1	1.03	**13.20**	**0.001**		29.16	3.40	0.069	
W x Small herb (SH)	1	0.01	0.08	0.781		55.84	**6.80**	**0.011**	
W x Tall herb (TH)	1	0.21	2.32	0.132		3.03	0.34	0.562	
W x Plot	75	0.09	1.13	0.270		8.84	0.72	0.947	
Year	1	36.62	**459.19**	**<0.001**	↑	2224.85	**180.10**	**<0.001**	↑
Year x SR	1	0.88	**11.07**	**0.001**		565.67	**45.79**	**<0.001**	
Year x FG	1	0.14	1.79	0.182		73.77	**5.97**	**0.016**	
Year x LE	1	0.04	0.49	0.484		661.36	**91.53**	**<0.001**	
Year x GR	1	0.04	0.55	0.459		432.74	**46.05**	**<0.001**	
Year x SH	1	0.01	0.11	0.745		38.24	3.22	0.075	
Year x TH	1	0.01	0.13	0.714		2.19	0.18	0.676	
Year x W	1	1.23	**15.40**	**<0.001**		10.34	0.84	0.362	
Year x W x SR	1	1.23	**15.42**	**<0.001**		0.39	0.03	0.859	
Year x W x FG	1	0.03	0.38	0.538		0.22	0.02	0.894	
Year x W x LE	1	0.21	2.63	0.107		122.29	**16.92**	**<0.001**	
Year x W x GR	1	0.05	0.65	0.421		29.70	3.16	0.077	
Year x W x SH	1	0.04	0.46	0.498		57.24	**4.82**	**0.030**	
Year x W x TH	1	0.16	1.99	0.161		3.40	0.27	0.603	
Year x W x Plot	150	0.08	**2.54**	**<0.001**		12.35	**2.94**	**<0.001**	
Residuals	468	0.03				4.21			

Model terms were fitted sequentially and tested against the respective residuals. Note that functional group identities were fitted as separate contrasts in series of analyses. Given are the degrees of freedom (df), mean sums of squares (MS), F ratios (F) and p values (p); significant effects are marked in bold. Arrows indicate increase (↑) or decrease (↓) of the variables with species richness, functional group number, presence of a particular functional group or time; SR = sown species richness, FG = functional group number.

Chapter 9

Experimental plant communities develop phylogenetically overdispersed abundance distributions during assembly

Allan, E., Jenkins, T., Fergus, A.J.F., Roscher, C., Fischer, M., Petermann, J.A., Weisser, W. & Schmid, B. 2013. *Ecology* 94: 465-477.

Abstract

The importance of competition between similar species in driving community assembly is much debated. Recently, phylogenetic patterns in species composition have been investigated to help resolve this question: phylogenetic clustering is taken to imply environmental filtering, and phylogenetic overdispersion to indicate limiting similarity between species. We used experimental plant communities with random species compositions and initially even abundance distributions to examine the development of phylogenetic pattern in species abundance distributions. Where composition was held constant by weeding, abundance distributions became overdispersed through time, but only in communities that contained distantly related clades, some with several species (i.e., a mix of closely and distantly related species). Phylogenetic pattern in composition therefore constrained the development of overdispersed abundance distributions, and this might indicate limiting similarity between close relatives and facilitation/complementarity between distant relatives. Comparing the phylogenetic patterns in these communities with those expected from the monoculture abundances of the constituent species revealed that interspecific competition caused the phylogenetic patterns. Opening experimental communities to colonization by all species in the species pool led to convergence in phylogenetic diversity. At convergence, communities were composed of several distantly related but species-rich clades and had overdispersed abundance distributions. This suggests that limiting similarity processes determine which species dominate a community but not which species occur in a community. Crucially, as our study was carried out in experimental communities, we could rule out local evolutionary or dispersal explanations for the patterns and identify ecological processes as the driving force, underlining the advantages of studying these processes in experimental communities. Our results show that phylogenetic relations between species provide a good guide to understanding community structure and add a new perspective to the evidence that niche complementarity is critical in driving community assembly.

Introduction

A major question in ecology is what drives community assembly. There is still much debate about the relative importance of limiting similarity or environmental filtering, with analyses based on functional traits giving different results in natural communities (Stubbs and Wilson 2004, Thompson et al. 2010). The evolutionary history of species has long been used to understand community assembly (e.g., Darwin 1859) but has recently received increased attention (Webb et al. 2002, Cadotte et al. 2008, Cavender-Bares et al. 2009, Vamosi et al. 2009). Using phylogenetic relations between species to understand their interactions has the advantage that phylogeny may integrate information on hard-to-measure traits, such as the co-evolved enemies shared between species, that would not be included in functional trait measures (Kraft and Ackerly 2010). Phylogenetic patterns in community composition can indicate the ecological processes underlying community assembly: phylogenetic overdispersion, where the species present are distantly related, is expected to arise from limiting similarity processes, which prevent closely related species from coexisting (Pacala and Tilman 1994). Phylogenetically clustered compositions, where the species present are closely related, are often interpreted as being caused by environmental filtering on phylogenetically conserved species traits (Vamosi et al. 2009). However, competition could also cause this pattern if competitive ability itself is phylogenetically conserved (Mayfield and Levine 2010). Quantifying the importance of competition for driving phylogenetic patterns is therefore important for understanding the mechanisms behind them.

The vast majority of studies on phylogenetic pattern have examined the presence/absence of species in a community (composition) but not their local abundances (Hardy 2008, Vamosi et al. 2009), meaning they have ignored later stages of community assembly. However, the species that dominate a community may not be a random sample, with respect to their functional traits, of those present (Cornwell and Ackerly 2010), and different processes can determine which species become abundant vs. those that establish at a site (Cingolani et al. 2007). Other studies have shown that different phylogenetic patterns may be found when incorporating data on species occurrence frequency (Kembel 2009, Kraft and Ackerly 2010) or abundance (Hardy and

Senterre 2007). Phylogenetic overdispersion may increase during succession (Webb et al. 2006, Letcher 2009) and some studies have shown a greater importance of environmental filtering in early successional communities (Helmus et al. 2010). Therefore species might shift their relative abundances during community assembly, so that the dominant species in a community become less closely related over time, i.e., abundance distributions become increasingly overdispersed.

Several studies have shown that the type of phylogenetic pattern found in a community depends on the phylogenetic scale: overdispersion should be more common in communities with close relatives present (Cavender-Bares et al. 2006, Swenson et al. 2006), where negative species interactions, i.e., competition, are expected to dominate (Burns and Strauss 2011). On the other hand, complementary (Cadotte et al. 2008, Gubsch et al. 2011) or facilitative (Valiente-Banuet and Verdu 2007) interactions may be more common between distantly related species. The presence of several distantly related clades in a community, each containing a number of species (a mix of closely and distantly related species), might therefore promote overdispersed abundance distributions. To test the influence of phylogenetic scale, the development of phylogenetic pattern in abundance distributions could be compared between communities containing species compositions fixed at different phylogenetic diversities, with the prediction that species abundances would only become overdispersed in communities containing distantly related clades each with several species (see Fig. 1). This idea of an interaction between phylogenetic pattern in community composition and the phylogenetic pattern in abundance distribution that develops has not yet been tested.

Allowing artificial plant communities to reassemble has been shown to lead to convergence in functional, species (Pfisterer et al. 2004, Fukami et al. 2005, Petermann et al. 2010) and phylogenetic diversity (Cadotte and Strauss 2011). A recent study showed that the species that established in reassembling communities tended to be either closely or distantly related to the residents (Cadotte and Strauss 2011). Allowing reassembly in composition to occur alongside assembly of abundances allows us to test whether communities converge on phylogenetic compositions that result in phylogenetically overdispersed abundance distributions. If communities converge at

overdispersed or clustered compositions there might be no phylogenetic pattern in abundance distributions (Fig. 1a, b) because phylogenetically based environmental filtering/limiting similarity has already occurred, so the strength of species interactions are not correlated with the phylogenetic distance between them. Alternatively, random phylogenetic compositions might result in overdispersed abundance distributions if closely related species can co-occur within a community but cannot both reach high abundance (Fig. 1c).

Biodiversity experiments provide an ideal opportunity to test these ideas because they contain replicate plots with a range of species numbers and compositions, the latter determined by a random draw from a species pool. It is therefore possible to study the development of phylogenetic pattern as these communities reassemble, while excluding local evolutionary or dispersal explanations (Cavender-Bares et al. 2009), something previous observational studies could not do. Here, we examine the development of phylogenetic patterns in the Jena Experiment, a grassland biodiversity experiment in Germany that manipulated species richness and functional group composition (Roscher et al. 2004). We calculated changes in phylogenetic pattern in abundance distributions for experimental communities over seven years, using a measure called abundance phylogenetic dispersion (APD; Hardy 2008), which quantifies whether the abundant species in a community are more or less closely related than the average. APD is a relative measure and is independent of phylogenetic pattern in composition, i.e., even communities composed of closely related species can in principle develop overdispersed abundance distributions.

We investigate changes in phylogenetic pattern in abundance distribution during two processes of community assembly or reassembly: first, using weeded communities with fixed species composition where phylogenetic pattern was only affected by changes in the relative abundances of species. Here we test for the interaction between phylogenetic pattern in species' presence/absence and the phylogenetic pattern in abundance distribution that develops. We also calculate the importance of interspecific competition for the development of phylogenetic pattern in abundance distribution by comparing the pattern expected for a community based on the abundance of its species in monoculture (i.e., without interspecific competition) with that observed in the

presence of interspecific competition. Using monocultures to infer the importance of complementary species interactions is the basis of the additive-partitioning method (Loreau and Hector 2001) and here we use an analogous approach to look at the importance of species interactions in driving phylogenetic pattern. Second, we investigate changes in phylogenetic pattern during the reassembly of communities following colonization by species from a common species pool; here species composition changes alongside abundances. Using the unique opportunity of a wide range of different plant communities composed of a common species pool and situated at a homogeneous field site, we examine the following hypotheses:

1. Over time communities become overdispersed in abundance and this is driven by interspecific competition.
2. In fixed-composition communities, the phylogenetic pattern in composition will affect the development of overdispersion in abundance distributions (see also Fig. 1).
3. Functional, and at the same time phylogenetic, groups with strong complementary interactions with other species (here: legumes) promote overdispersion.
4. Allowing species composition to reassemble along with changes in abundance will result in convergence in phylogenetic diversity and overdispersion in abundance distributions.

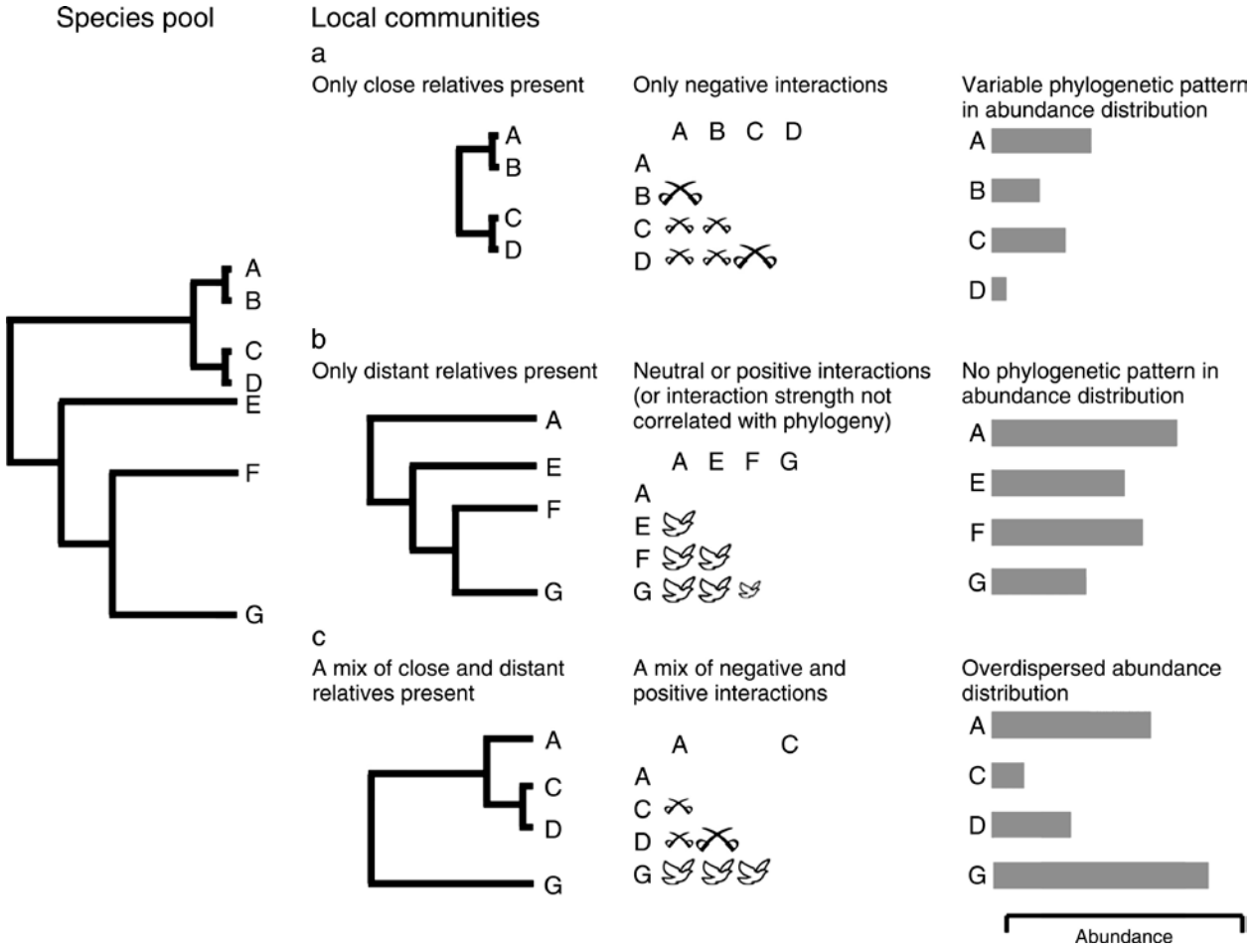

Fig. 1. The hypothesized effect of phylogenetic pattern in species' presence/absence in a community on the emergence of phylogenetic pattern in abundance distribution. The phylogeny of the species pool is shown on the left. Species have been assembled from the pool into three types of communities: (a) a community with clustered composition, i.e., only close relatives present; (b) a community with overdispersed composition, i.e., only distant relatives present; and (c) a community with random phylogenetic composition, containing distantly related clades but with some clades having multiple species, i.e., a mix of close and distant relatives. Hypothesized species interactions are shown on a matrix for each community: crossed swords represent negative interactions and doves represent positive or neutral interactions, larger symbols show stronger interactions. The consequences of these interactions for species abundances are shown on the right in bar plots. Where the composition is clustered or overdispersed (communities a or b) then the abundance distribution is less likely to be determined by phylogenetic relations between species because environmental filtering/limiting similarity has already determined community composition, and therefore the phylogenetic distances between species present do not predict the strength of their interactions. If the composition is phylogenetically random (community c) then there is more scope for species abundances to shift to reduce negative interactions between close relatives, and overdispersion in abundance distributions can develop.

Methods

The Jena Experiment

The Jena Experiment (see Plate 1) has 78 large plots (20 × 20 m) with 1, 2, 4, 8, or 16 plant species, selected from a pool of 60 plant species. Plants belong to four functional groups (FG): grasses, legumes, small herbs, and tall herbs (Roscher et al. 2004). Number and presence of FGs was varied systematically, e.g., plots with four or more species could have 1, 2, 3, or 4 FGs. In addition, monocultures of each species were grown on 3.5 × 3.5 m plots. Plots were sown with 1000 seeds/m2, equally divided among the species present and adjusted to species germination rates (Roscher et al. 2004). In this fixed-composition experiment species composition was held constant (except for extinctions) through biannual weeding in early April and July, when all species not sown into the plot initially were removed. Individual species-cover data were collected twice yearly, in spring and summer, on a subplot of 3 × 3 m. Biomass was harvested at the same time and sorted to species. For more details on sampling see Weigelt et al. (2010). For the main analyses here we use cover data from 2002–2009. Using cover means we estimate species abundances over a larger area than biomass sampling would have allowed, providing a better estimate of the abundance of less common species. For our calculations, we used only plots containing 4–16 species (46 plots) as it is not meaningful to calculate a phylogenetic pattern in the abundance of only one or two species.

We also investigated phylogenetic pattern in a reassembly experiment carried out in all 78 plots; monocultures and two-species plots were included because species numbers rapidly increased during reassembly (Roscher et al. 2009a). In this experiment, seeds of all 60 species were sown, in April 2005, at equal proportions and at a total density of 1000 viable seeds/m^2, into the existing vegetation in subplots of 2.00 × 2.25 m. Species not belonging to the species pool continued to be weeded out from July 2005. Cover estimations were made on the whole area of these subplots at the same time as in the large plots, using an identical protocol. Pre-2005 data came from two subplots of the same size weeded as the large plots (Roscher et al. 2009b).

Plate 1. Aerial view of the Jena Experiment in June 2006. The 20 × 20 m plots of the fixed composition experiments are clearly visible. The subplots of the reassembly experiment, opened to colonization in April 2005, are visible as differently shaded squares within some of the large plots. Photo credit: Alexandra Weigelt.

Phylogeny reconstruction

We searched GenBank in March 2009 and again in June 2012 for four gene sequences commonly used in building angiosperm phylogenies (Benson et al. 1999). We used closely related congeners for 2 of the 60 species for which there were no available sequence data (see Fig. 2). Each species used for the phylogeny reconstruction had sequence data for at least one gene and we had data for *rbcl* (90% of species), *matk* (97% of species), *5.8s* (75% of species), and *its2* (92% of species), resulting in a total sequence length of 3581 base pairs. Sequences were individually aligned for each gene, separately per plant family, in MUSCLE (Edgar 2004). We used jModeltest (Posada and Crandall 1998) to test for models of DNA substitution for each gene separately, resulting in the selection of GTR + Γ.

We performed dated Bayesian reconstructions and estimates of divergence times using BEAST version 1.7.2 (Drummond and Rambaut 2007), with *Amborella trichopoda* and *Magnolia grandiflora* as outgroups. To obtain a dated molecular

phylogeny we used six fossils: for the root of the tree (all angiosperms) and for the following groupings according to the Angiosperm Phylogeny Group tree (APG III 2009): Eudicots, Asterids, Rosids, Apiales and Fabaceae (Appendix A: Table A1). Parameters were estimated using two independent Markov chain Monte Carlo (MCMC) chains, each run for 26 million generations, and sampled every 1000 generations. Analyses were partitioned across the two mitochondrial genes, but the same site model was used for the two nuclear genes. We used a relaxed molecular clock model allowing branch lengths to vary according to an uncorrelated lognormal distribution and a Yule speciation tree prior. Convergence and burn-in was assessed using Tracer version 1.5 (Drummond and Rambaut 2007), by inspection of parameter values and their associated likelihoods and by estimation of effective sample size (ESS) (ESS > 200 indicates convergence; Drummond et al. 2006). Tree files were combined using TreeAnnotator version 1.4.8 (Drummond and Rambaut 2007), with the first 10% of trees discarded as burn-in, in order to produce a posterior distribution of trees. Outgroups were removed from the trees and 10% of those in the posterior distribution (4680) were used in subsequent analyses.

Sown phylogenetic diversity

In order to test the interaction between phylogenetic pattern in composition and the development of phylogenetic pattern in abundance distributions (Fig. 1), we calculated two measures of phylogenetic diversity based on the sown species composition of the plots in 2002. *Mean pairwise distance* (MPD) measures the mean phylogenetic distance between all pairs of species and is affected by the number of deeper splits in the phylogeny. *Mean nearest neighbor distance* (MNND) measures the mean distance between each species and its closest relative and measures dispersion at the tips of the phylogeny (Webb et al. 2002). Both were calculated using picante (Kembel et al. 2010) in R 2.10 (R Development Core Team 2010) for each plot using 4680 trees. In order to account for uncertainties in phylogenetic reconstruction, median as well as lower (25%) and upper (75%) quartile values were calculated.

Sown MPD and MNND were significantly positively correlated across communities; but several had high MPD and low MNND (Fig. 3a), indicating the presence of distantly related clades each with several species, i.e., a mix of close and distant relatives. MPD was unrelated to species richness, while MNND was lower in species-rich communities (Appendix C: Fig. C1). The correlations between sown phylogenetic and species diversity provide the motivation for fitting all factors in our models (see *Statistical analysis*, below): we always fitted sown MPD and MNND as explanatory variables, meaning they did not change over time and were not affected by any species losses.

Phylogenetic pattern based on species abundance distributions

We calculated phylogenetic pattern in abundance distributions using APD (Hardy 2008), which quantifies the extent to which closely related species have similar abundance. For details on the calculation of this metric see Appendix B: Eqs. B.1 and B.2, or Hardy (2008). An APD <0 means overdispersed abundance distributions because it indicates that the most abundant species are more distantly related to each other than are the average pair of species. An APD >0 indicates clustered abundance distributions, meaning abundant species are closely related. As APD is a relative measure, even communities with low MPD can show overdispersed abundance.

For the fixed-composition and the reassembly experiments we calculated APD for all plots, years, and cover surveys, using 4680 trees, and median as well as lower (25%) and upper (75%) quartile values were obtained. The APD was calculated relative to MPD between all sown species in a plot. In order to test for significant phylogenetic pattern across plots we tested whether average APD significantly differed from 0; if average APD across plots did not significantly differ from 0 we considered them to have random phylogenetic structure in abundance.

Effect of interspecific competition on phylogenetic pattern

In order to test for the importance of interspecific competition in driving phylogenetic pattern, we used monoculture data to calculate a measure we call "$D^{\mathrm{B}}_{\mathrm{diff}}$"; for details on the calculation of this metric see Appendix B and Eq. B.3. The "$D^{\mathrm{B}}_{\mathrm{diff}}$" will be negative if the most abundant species in mixture are more phylogenetically distant from each other

than are the most abundant species in monoculture, this would suggest that competition drives overdispersion in abundance. The "D^{B}_{diff}" will be positive if the most abundant species in mixture are more closely related than are the most abundant species in monoculture; this would indicate that interspecific competition results in phylogenetically clustered compositions.

Statistical analysis

To study effects of sown phylogenetic diversity, species richness and FG composition on change in phylogenetic pattern over time we used linear mixed-effects models fitted with the lme4 package in R (Bates and Sarkar 2007). The same model was used for data from the fixed-composition and the reassembly experiments. Models included a random effect for plot (46 plots in the fixed composition and 78 plots in the reassembly experiment) and a random effect for cover survey coded as a categorical factor (15 time points for the fixed composition and 6 time points for the reassembly experiment). Fixed effects were: time (continuous variable), sown species richness (log-transformed), sown MPD and sown MNND, FG composition (fitted as the presence/absence of each FG), and interactions between these terms (Appendix C: Tables C1 and C2). We simplified full models by removing nonsignificant terms and used likelihood-ratio tests to compare models with and without the term of interest (Crawley 2007).We tested for an effect of season by comparing a model with season and interactions between season, species richness, phylogenetic diversity, and FG composition (23 terms) with a model without any season terms (15 terms). In all cases the simpler model was preferred and seasonal effects are therefore not considered further. To test for a main effect of time we compared a model with a linear continuous term for time as the only fixed effect, with an intercept-only model. We analyzed the change in "D^{B}_{diff}" over time in mixed models with the same random effects as above and with fixed effects for time and interactions between time and sown species richness, sown MPD, and sown MNND.

To examine whether plots had random phylogenetic structure we fitted intercept-only models to test whether mean values across plots differed from 0. We did this for

each time period (cover survey or biomass harvest) for APD values in the reassembly and fixed-composition and for "D^{B}_{diff}" in the fixed-composition experiment.

We repeated all analyses using APD, MPD, and MNND values from 25% or 75% quartiles to correct for phylogenetic uncertainty. This led to the same qualitative results.

Results

Phylogeny reconstruction

The phylogenetic reconstructions and the divergence time estimations converged in the same likelihood space and gave well-supported trees that agreed with the Angiosperm Phylogeny Group (APG) III classification (APG III 2009) (see Fig. 2 for the maximum clade credibility tree).

Phylogenetic pattern in the fixed-composition experiment

Species had initially been sown at equal proportions, so at sowing APD (abundance phylogenetic dispersion) = 0. In the fixed-composition experiment, phylogenetic pattern in abundance distributions changed over time ($\chi^2 = 24.0$; $P < 0.001$): communities had developed clustered abundance distributions by the first cover survey (2002, APD = 0.09 ± 0.03 [mean ± SE]; $P < 0.01$) but after five years average APD was negative, i.e., abundant species were on average less closely related to each other than less abundant ones.

Phylogenetic pattern in species composition—sown MPD (mean pairwise distance) and sown MNND (mean nearest neighbor distance)—affected the development of phylogenetic pattern in abundance distributions (Appendix C: Table C1). In order to visualize these effects, communities were divided into three groups: those sown with only close relatives (low MPD and MNND), those sown with distantly related but species-rich clades (a mix of close and distant relatives; low MNND but high MPD) and those sown without close relatives (high MPD and MNND) (Fig. 3a). In communities with only closely related species APD decreased over time ($\chi^2 = 8$; $P < 0.01$) but there was large variability between plots and they ended up with, on average, random phylogenetic structure in abundance distributions (Fig. 3b). In communities containing distantly related but species-rich clades, APD decreased over time ($\chi^2 = 33$; $P < 0.001$) and abundance distributions were overdispersed by the end of the time

series (Fig. 3c). In communities with only distantly related species, APD did not change over time ($\chi^2 = 2.7$; $^{P} = 0.09$) and phylogenetic pattern in abundance remained random (Fig. 3d).

Functional group composition affected the development of phylogenetic pattern in abundance distributions as communities with legumes became more rapidly overdispersed (Appendix C: Table C1). There were also significant interactions between presence of grasses, small herbs, tall herbs and time (Appendix C: Table C1). Plots with small herbs had lower APD values at the beginning of the experiment but higher APD at the end. Plots with grasses and plots with tall herbs were less overdispersed at the end of the experiment. The functional composition, as well as the phylogenetic diversity, of the community therefore affected the degree of overdispersion. The species richness of the community had no effect on the development of phylogenetic pattern in abundance distributions although species-rich plots were slightly less overdispersed (Appendix C: Table C1).

"$D^{\mathrm{B}}_{\mathrm{diff}}$" (which compares the observed phylogenetic pattern in abundance distribution for a community with that expected based on the monoculture abundances of the species) decreased over time, which is what would be predicted if competition drove the increase in overdispersion ($\chi^2 = 24$; $P < 0.001$; Appendix B: Fig. B1). This pattern was strongest in the 16-species plots (sown diversity × time interaction, $\chi^2 = 19$; $P < 0.001$). At the beginning of the experiment was significantly greater than 0, indicating that the abundant species in mixture were more closely related to each other than were the most abundant species in monoculture: i.e., interspecific competition resulted in phylogenetically clustered abundance distributions. By the end of the time series was significantly smaller than 0, meaning abundant species were more distantly related in mixture than in monoculture. This indicates that interspecific competition drove an increase in phylogenetic overdispersion in abundance distributions.

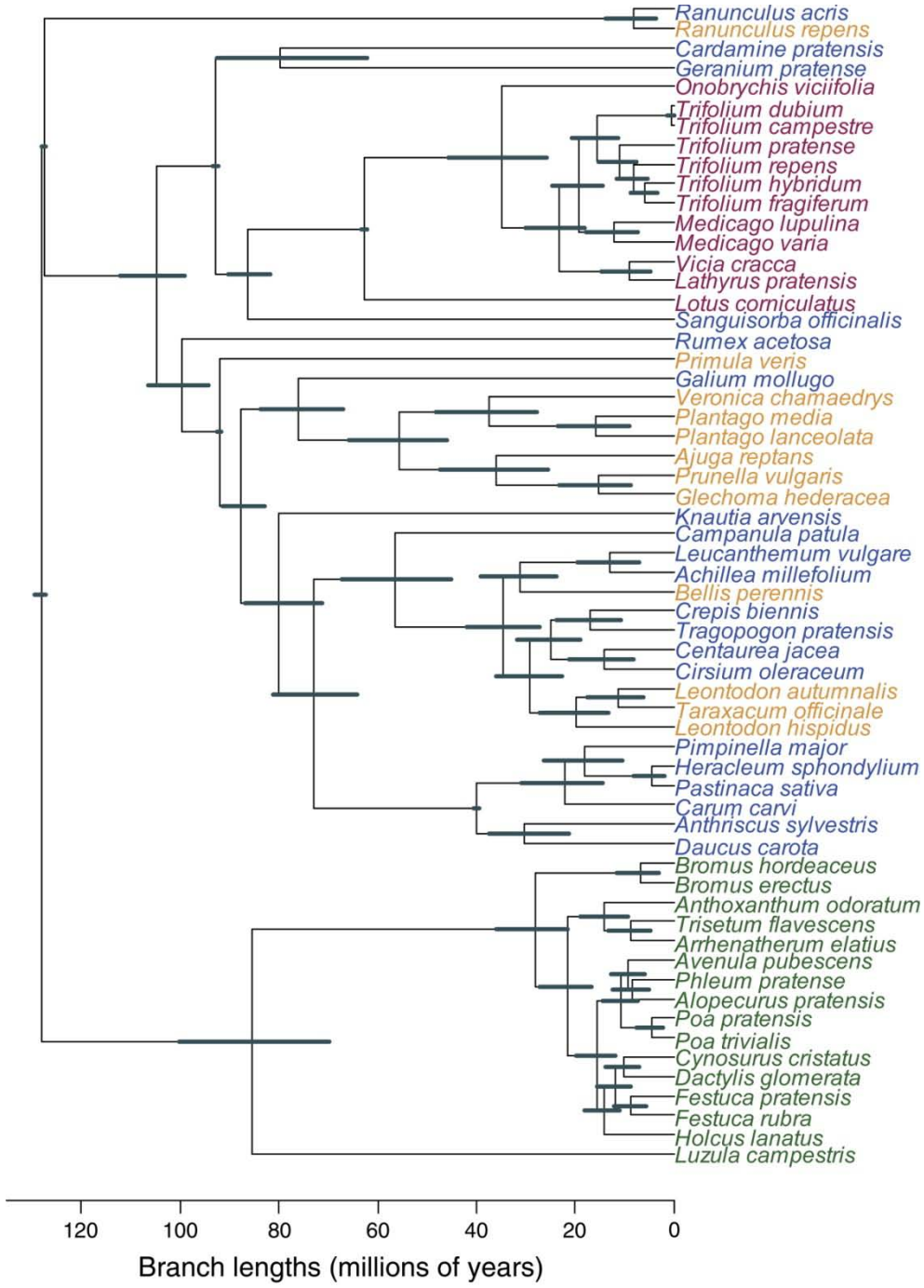

Fig. 2. Maximum clade-credibility phylogeny of the 60 species in the Jena Experiment. Different functional groups are differently colored: graminoids in green, legumes in yellow, small herbs in red, and tall herbs in blue. The 95% confidence intervals for node ages are shown. Congeners were used for *Onobrychis viciifolia* (*O. montana*) and for *Pimpinella major* (*P. saxifraga*). Node support was high: 66% of nodes gave a posterior probability of 1, and a further 23% a posterior probability >0.97. Only seven nodes were less well supported, four in the Poaceae, plus the placement *of Bellis perennis* (0.64), *Rumex acetosa* (0.68), and the node between *Cardamine pratensis* and *Geranium pretense* (0.82).

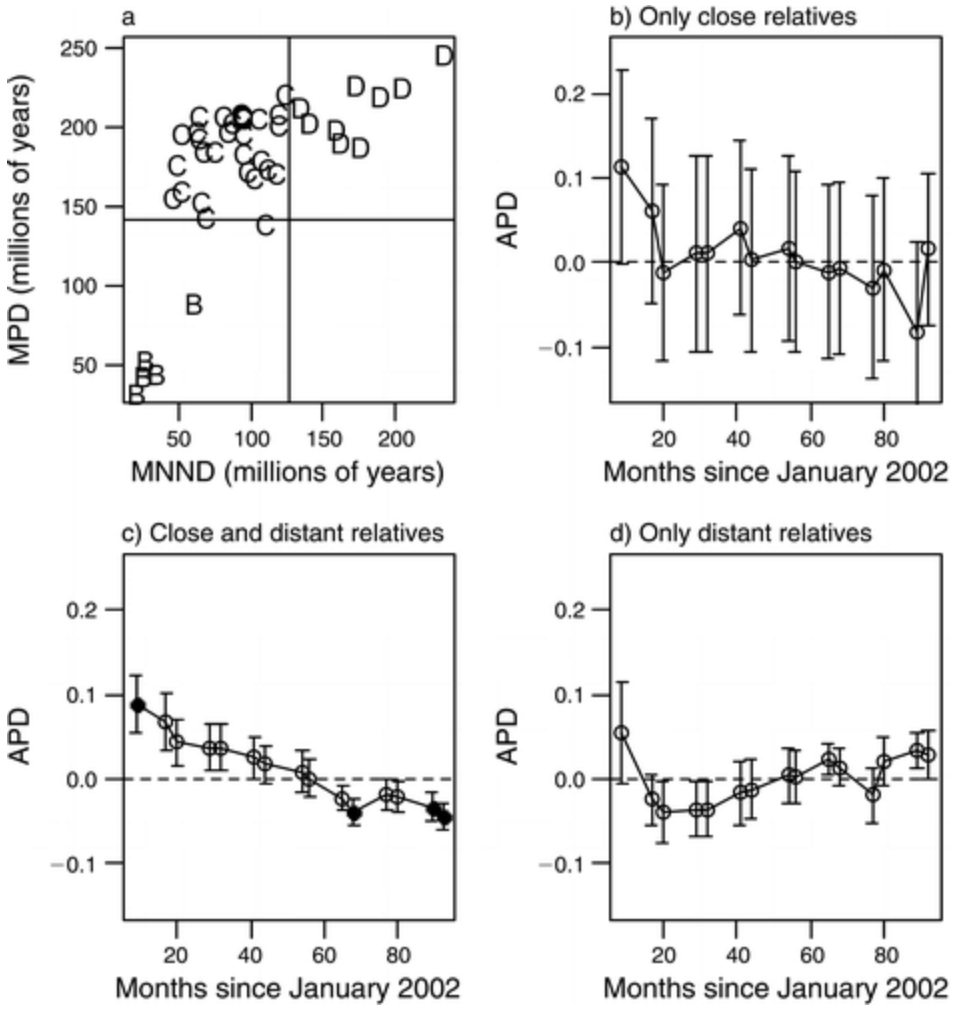

Fig. 3. Change in abundance phylogenetic dispersion (APD) over time in the fixed-composition experiment. Negative values of APD indicate overdispersed abundance distributions, and positive values indicate clustered abundance distributions. (a) Relationship between sown MPD (mean pairwise distance) and MNND (mean nearest neighbor distance) across plots. Plots were classified into three groups based on sown phylogenetic diversity: (b) those with MPD < 141 million years (Myr) and MNND < 126 Myr; (c) MPD > 141 Myr and MNND < 126 Myr; and (d) MPD > 141 Myr and MNND > 126 Myr. In panel (a) these groups are indicated by uppercase letters (B–D); cut-off points (solid lines) are the midpoints in the range of MPD or MNND values and were used for illustration only; MPD and MNND were analyzed as continuous variables. In panels (b)–(d) open circles show plots with random phylogenetic structure (APD not significantly different from 0), and solid circles show those with significant phylogenetic structure (APD significantly different from 0). Data are means ± SE; significance is at the 5% level.

Phylogenetic pattern in the reassembly experiment

In the reassembly experiment, communities became, on average, clustered in the first two surveys after colonization (spring 2005, APD = 0.06; $P = 0.01$; and autumn 2005, APD = 0.04; $P = 0.05$) and overdispersed in abundance in spring 2006 (APD = −0.02; $P = 0.02$). In subsequent surveys plots were not significantly overdispersed on average; however this was due to a single former legume monoculture that became entirely dominated by grasses and therefore highly clustered in abundance relative to all species that could colonize the community (see Fig. 4a). Excluding this plot, communities were also overdispersed in autumn 2007 (APD = −0.02; $P = 0.01$). Calculating APD ignoring phylogenetic pattern in the colonizing species led to similar patterns, but there was stronger evidence for overdispersion from spring 2006 onward and weaker evidence for clustering immediately after colonization (see Appendix D: Fig. D1).

In the course of the experiment, communities converged in phylogenetic diversity. By spring 2007 the range of APD values had contracted (Fig. 4a). MPD and MNND converged more rapidly: at convergence MPD was higher but MNND was lower than mean sown values (Fig. 4b and c). Therefore species composition converged so that plots ended up containing distantly related but species-rich clades.

The originally sown phylogenetic diversity of the communities affected the change in phylogenetic pattern immediately after colonization (Appendix C: Table C2). To explore this result, plots were classified as having sown (in 2002) MPD and MNND higher, lower, or in the range of 95% of values to which communities had converged by summer 2007 (Fig. 4b, c). MPD and MNND increased following colonization on plots with sown MPD or MNND lower than converged values and MPD and MNND decreased following colonization on plots with sown MPD and MNND higher than converged values. Classifying plots by sown MPD and MNND gave seven combinations but factor-level reduction (in a mixed model testing for differences in the slope of APD over time for these different categories) led to four categories, and change in phylogenetic pattern was then analyzed in these groups of communities separately. (1) Seven plots had been sown with only close relatives (sown MPD and MNND lower than converged values): here APD became strongly clustered after invasion and then decreased (time

effect $\chi^2 = 8$; $P < 0.01$) (Fig. 4d). (2) Nineteen plots had been sown with some more distantly related species (sown MPD lower; sown MNND higher or in the range of converged values) and these became less clustered after invasion than the first set of plots, before APD decreased (time effect $\chi^2 = 9$; $P < 0.01$) (Fig. 4e). (3) Nineteen plots had been sown with distantly related but species-rich clades (MPD and MNND in the range of converged values) and here phylogenetic pattern was random after colonization and then decreased (time effect $\chi^2 = 10$; $P < 0.01$) (Fig. 4f). (4) Seventeen plots had been sown with mostly distantly related species (sown MPD higher and MNND in the range of converged values), these initially became strongly overdispersed and then APD increased (time effect, $\chi^2 = 11$; $P < 0.01$) (Fig. 4g). This last result suggests that APD at convergence is lower than the maximum possible.

Development of overdispersion following colonization was also affected by sown species richness and functional group composition: in the first year overdispersion developed on 1-, 2- and 4-species communities, but 8- and 16-species communities became clustered (Appendix C: Table C2). Legume presence also promoted the development of overdispersion (Appendix C: Table C2).

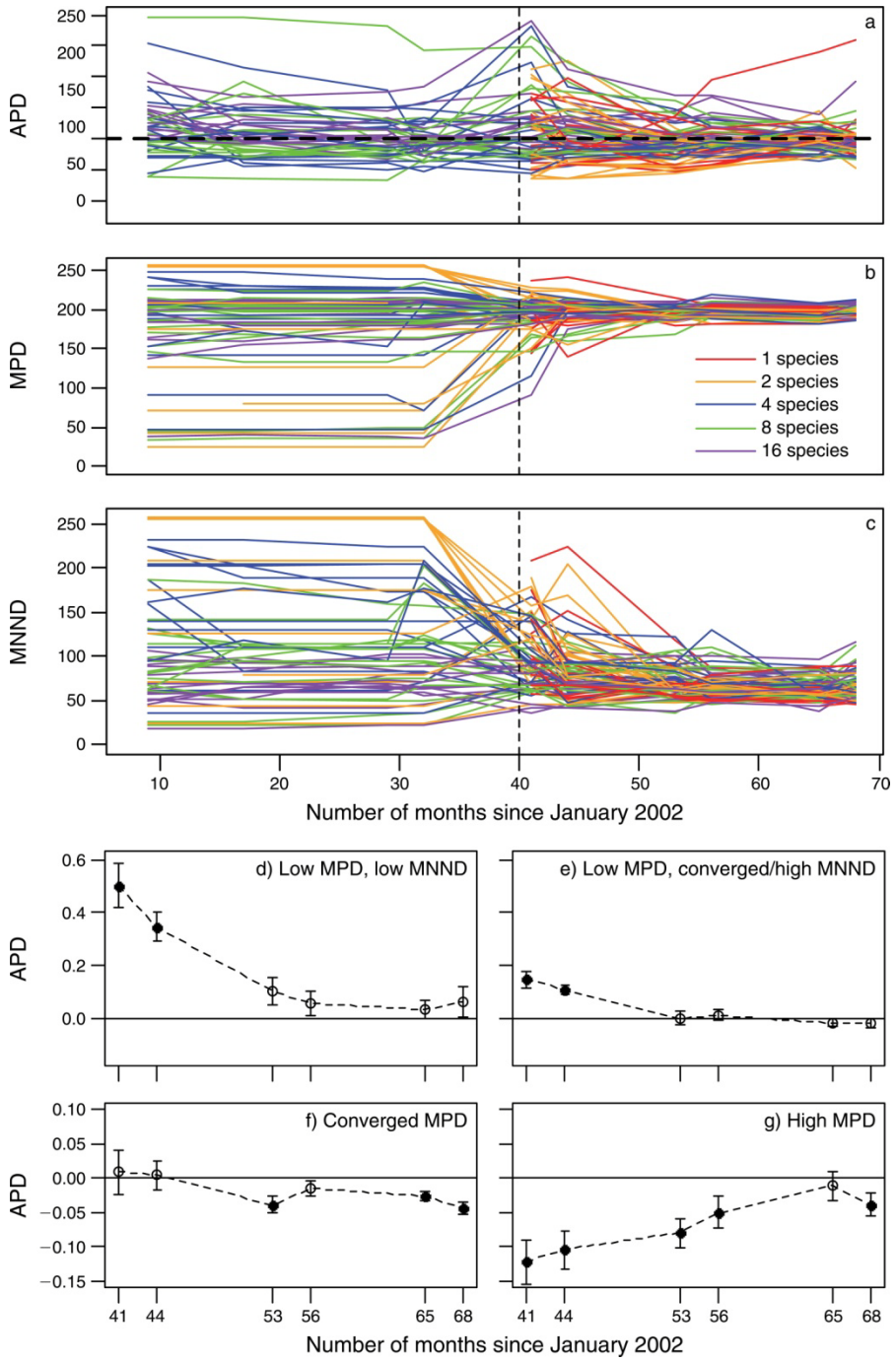

Fig. 4. (a–c) Change in phylogenetic pattern in the reassembly experiment. Colonization was allowed from April 2005 (time = 40). Convergence, following colonization, in: (a) APD; (b) realized MPD (mean pairwise distance) [by summer 2007 95% of plots had MPD between 189 and 209 million years (Myr)]; and (c) realized MNND (mean nearest neighbor distance) [by summer 2007, 95% of plots had MNND between 47 and 97 Myr]. Each line in panels (a)–(c) represents one plot: different sown diversities are colored differently.

Fig. 4 (continued) (d–g) Mean change in APD following colonization for plots classified according to their sown MPD and MNND (in 2002) relative to 95% of the MPD and MNND values to which plots converged in summer 2007; sown values are therefore starting values and, realized phylogenetic diversity changed following colonization. Data are means ± SE; significance is at the 5% level. (d) Sown MPD < 189 Myr, and sown MNND < 47 Myr; (e) sown MPD < 189 Myr, and sown MNND > 47 Myr; (f) sown MPD > 189, and <209 Myr (sown MNND > 47 Myr); and (g) sown MPD > 209 Myr (sown MNND > 47 Myr). Open circles show plots with random phylogenetic structure (APD not significantly different from 0), and solid circles show those with significant phylogenetic structure (APD significantly different from 0). Note the y-axis scale in panels (d) and (e) is different from that in panels (f) and (g).

Discussion

Change in phylogenetic pattern in communities with fixed species composition

Phylogenetic overdispersion in abundance distributions developed in experimental communities with fixed species compositions, as predicted by our first hypothesis. The relative abundances of the species in these communities differed from the ones expected based on their performance in monoculture, and by the end of the time series the dominant species in mixture were less closely related to each other than expected based on their abundance in monoculture. This would suggest that interactions between species, perhaps limiting similarity and/or facilitation, drove the increase in phylogenetic overdispersion in abundance distributions. Environmental filtering has also been shown to cause overdispersion (Cavender-Bares et al. 2006), however this would be an unlikely explanation for our results given that by the end of the time series the abundant species in polyculture communities were more distantly related to each other than were the most abundant species in monoculture. If environmental filtering drove overdispersion then the same species should become abundant in monoculture. If mixtures because all experimental communities were grown in the same environment.

Several mechanisms may have caused the development of overdispersed abundances. Phylogenetically related species may have shared ecological niches, meaning that close relatives could not coexist at high abundance over time (Maherali and Klironomos 2007). Closely related species could share similar resource (Prinzing 2001, Cahill et al. 2008) or pathogen niches (Gilbert and Webb 2007) and therefore compete more strongly with each other (Burns and Strauss 2011). More distantly related species, in contrast, may have had complementary or facilitative interactions,

which is supported by the finding that fixed-composition plant communities with higher phylogenetic diversity produce more biomass (Cadotte et al. 2008). Previous work has shown an increase in species complementarity effects over time in biodiversity experiments (Cardinale et al. 2007, Marquard et al. 2009) and observational studies on succession have also shown an increase in overdispersion through time (Letcher 2009). The increase in overdispersion through time that we find, and the increasing phylogenetic diversity in mixtures vs. monocultures, indicates that limiting similarity became increasingly important as the communities assembled.

Not all communities became phylogenetically overdispersed: the development of overdispersed abundance distributions depended on the phylogenetic pattern in composition (hypothesis 2). Communities lacking close relatives did not develop overdispersed abundance distributions. These results support those of studies that have found a stronger pattern of overdispersion at small phylogenetic scales (Cavender-Bares et al. 2006, Swenson et al. 2006). Strong negative interactions may only occur between close relatives: for example, Gilbert and Webb (2007) found that the ability of the fungal pathogens of one plant species to attack another declined with phylogenetic distance between the plants, but the decline was steepest between the closest relatives. Competitive exclusion was also shown to occur more frequently and more rapidly between closely related protist species (Violle et al. 2011). In communities without close relatives present, phylogenetic distance between species may not have been a good predictor of their interactions.

Although communities without close relatives did not develop overdispersion, neither did communities composed of plants from only one family, i.e., pure grass or legume communities. These communities could in principle develop overdispersed abundance distributions because our measure of overdispersion was calculated relative to the composition of the community. There was also large variability between communities with only close relatives present, suggesting that some did develop overdispersed abundance distributions. In a microbial system functionally similar species had antagonistic interactions with each other, meaning that increasing the number of functionally similar species in a community reduced ecosystem function (Jousset et al. 2011). In our single-family communities, it is therefore conceivable that

all interactions were competitive and this led to a large variability in phylogenetic pattern of abundance distributions. In communities that did develop overdispersion, positive interactions, either complementary or facilitative (Valiente-Banuet and Verdu 2007), among distantly related species may have been important. Perhaps only in communities with both close and distant relatives, where a mix of positive and negative interactions might be expected, could species abundances shift to reduce negative interactions between close relatives.

Phylogenetic diversity effects were independent of species richness: the development of overdispersed abundance distributions was not affected by species number. Greater overdispersion in species-rich communities might be expected if there is greater complementarity in these communities (Marquard et al. 2009); however our results show that phylogenetic diversity is more important than species richness in driving these patterns. More diverse plots did, however, develop a greater difference in phylogenetic diversity between mixture and monocultures.

Functional complementarity was probably an important mechanism underlying the development of phylogenetic overdispersion (hypothesis 3) because legume presence significantly increased overdispersion. There are two possible reasons why legumes increased overdispersion: (1) they can facilitate other species by increasing soil fertility (Temperton et al. 2007, Gubsch et al. 2011) and (2) they may be particularly sensitive to taxon-specific pathogen accumulation and/or phosphorus depletion (Roscher et al. 2011), preventing their dominance over time. However, complementarity between functional groups cannot be the only reason that phylogenetic overdispersion increases over time: after correcting for functional group presence in our statistical models, the development of overdispersion in abundance distributions still depended on sown phylogenetic diversity.

All experimental communities had phylogenetically random abundances when sown but differential recruitment and time needed for establishment (Heisse et al. 2007) led to clustered abundance distributions by the first cover survey. Traditionally, clustered patterns were thought to arise from environmental filtering on conserved species traits, and early successional or disturbed communities have been shown to be dominated by closely related species (Helmus et al. 2010) because regeneration traits

are phylogenetically conserved (Burns and Strauss 2011). However by the first cover survey, the species that had become abundant in mixture were more closely related to each other than were the abundant species in monoculture. Interspecific competition may therefore have caused the increase in clustering at the beginning of the experiment (Mayfield and Levine 2010). To fully test this idea would require assessing phylogenetic signal in fitness or competitive ability measures for all species. An analysis of several commonly measured morphological and physiological traits showed that most had low phylogenetic signal (D. F. B. Flynn, E. Allan, T. Jenkins, C. Roscher, and B. Schmid, unpublished manuscript) but there were no direct measures of fitness traits. This suggests that phylogenetic distance quantifies variation in unmeasured traits. Consistent with increasing complementarity effects over time in the Jena Experiment (Marquard et al. 2009), our results suggest that only highly competitive species were able to persist initially, before niche differences became important in driving coexistence in later years.

Change in phylogenetic pattern following colonization of new species

Communities that were allowed to reassemble through colonization of new species also became overdispersed (hypothesis 4). Colonization of communities led to a convergence in phylogenetic diversity after three years, agreeing with some other recent results (Cadotte and Strauss 2011). Like Cadotte and Strauss (2011), we found that communities ended up with species-rich but distantly related clades. This suggests that community composition did not become phylogenetically overdispersed, although abundance did. This idea is further supported by the fact that overdispersion in abundance was as evident when it was calculated ignoring phylogenetic pattern in composition. Such results show the importance of looking at phylogenetic pattern in abundance distributions because they imply that closely related species can co-occur in a community but cannot both reach high abundance.

The sown phylogenetic pattern in composition did affect the change in phylogenetic pattern in abundance distributions, following colonization. Communities originally sown with only closely related species developed clustered abundance distributions immediately after colonization. This was because resident species remained dominant in the first year and these were clustered relative to the colonizers,

which were still at low abundance. In some of our colonized communities, those originally lacking close relatives, overdispersion in abundance distributions briefly increased above the level to which all communities later converged. This result suggests that processes other than limiting similarity may be important in determining the identity of the species in these communities. A balance between environmental-filtering and limiting-similarity processes or between competitive ability differences and niche differences (Mayfield and Levine 2010) may therefore have led to overdispersion at convergence being lower than the maximum possible.

Conclusions

We found evidence for the development of overdispersion in abundance distributions in our experimental grassland communities, and were able to show that this was driven by interspecific competition, which suggests that limiting similarity processes become increasingly apparent as these communities reassemble. If the composition of the community was held constant, the emergence of phylogenetic overdispersion in abundance distributions depended on the presence of species-rich but distantly related clades in the community. Interactions between both closely related and distantly related species may therefore have driven the emergence of overdispersed abundance distributions. Allowing composition to also reassemble resulted in convergence in phylogenetic diversity and in communities that were composed of several distantly related but species-rich clades and that had overdispersed abundance distributions. This suggests that limiting similarity processes determine which species dominate a community but not which species occur in a community. Crucially, as our study was carried out in experimental communities, we can rule out local evolutionary or dispersal explanations for these patterns and identify ecological processes as the driving force, underlining the advantages of studying these processes in experimental communities. Phylogenetic relations between species may provide a good guide to their interactions because they integrate information on hard-to-measure traits such as pathogens shared between species that would not be included in studies based on functional traits. Our results show the importance of considering phylogenetic relations between species to

understand community structure, moreover, they add a new perspective to the evidence that niche complementarity is critical in driving community assembly.

Acknowledgments

We thank Nicolas Salamin, Charlotte Ndiribe, and Gavin Thomas for help and advice on phylogeny reconstruction, and Dan Flynn and Tadashi Fukami for comments on an earlier version of the manuscript. We thank Elisabeth Marquard for providing some cover data and Alexandra Weigelt for her management of the Jena Experiment. We also thank the gardeners and technical staff for maintaining the site, weeding, mowing, and data collection. Many student helpers were also involved in the weeding of the experiment. This project was funded by the Deutsche Forschungsgemeinschaft (FOR 456) with additional contributions from the Swiss National Science Foundation (grant 31003A-107531 to B. Schmid).

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Appendix A. A table presenting information about the angiosperm fossils used in the molecular phylogenetic tree calibration.

Table A1. The angiosperm fossils used in the molecular phylogenetic tree calibration. A log-normal distribution (mean = 1.0, SD = 0.1) was used for each fossil calibration and the prior distributions only differed by the offset (estimated age of grouping). CG = Crown group and SL = stem age.

Calibration point	**Fossil**	**Fossil age** (Myr)	**Hard lower bound / median / upper 95% CI used**	**Reference**
CG	Angiosperm	130	132.2 / 132.7 / 133.3	(Magallón and Castillo 2009)
CG	Eudicots	125	127.2 / 127.7 / 128.3	(Magallón and Castillo 2009)
CG	Asterids	89.3	91.53 / 91.57 / 92.61	(Martínez-Millán 2010) (Magallón and Castillo 2009)
CG	Rosids	90	93.23 / 93.72 / 94.31	(Wang et al. 2009)
CG	Apiales	37.2	39.4 / 39.9 / 40.5	(Magallón and Castillo 2009)
SL	Fabaceae	37.2	39.4 / 39.9 / 40.5	(Lavin et al. 2005)

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Appendix B. Details on the calculation of APD and D^B_{diff} and change in D^B_{diff} over time in the fixed composition experiment.

Calculation of APD

To calculate phylogenetic dispersion in abundance distributions we used APD (Hardy 2008). To calculate this metric an abundance-weighted measure of MPD, D^B, is calculated following Eq. B.1:

$$D^B = \frac{\sum_i^n \sum_{j \neq i}^n f_i f_j PD_{ij}}{\sum_i^n \sum_{j \neq i}^n f_i f_j}$$

where f_i is the relative abundance of the *i*-th species, f_j is the relative abundance of the *j*-th species and PD_{ij} is the phylogenetic distance between them. The measure of D^B is then compared to the (non-abundance-weighted) MPD of all species in the community according to Eq. B.2.

$$APD = \frac{MPD - D^B}{MPD}$$

D^B = MPD (APD = 0) indicates random phylogenetic structure in abundance distributions; D^B > MPD (APD < 0) indicates phylogenetic overdispersion and D^B < MPD (APD > 0) indicates phylogenetic clustering. In our analyses we used sown MPD values to calculate APD, however in the re-assembly experiment we also calculated APD based on the realised MPD in the plot, see Appendix D.

Effect of interspecific competition on phylogenetic pattern in abundance distributions: calculation of D^B_{diff}

We quantified the effect of interspecific competition on phylogenetic pattern in abundance distributions by comparing species abundances in monoculture and in mixture. We used biomass for this analysis because, although cover gives a good estimate of species relative abundances, comparing absolute cover on monocultures would be problematic: many monocultures reach 100% cover whilst differing strongly in biomass. We used the monoculture biomasses of each species to create communities with the same composition as the 46 polyculture communities but with the biomass of the species coming from monoculture, i.e. each species had the biomass it would attain without interspecific competition. We did this separately for each biomass harvest. We then calculated $D^B$for these simulated "monoculture communities" (D^B_{mono}) and for the real mixture communities, also using biomass, (D^B_{mix}) and derived the relative difference in D^B, according to Eq B.3:

$$D^B_{diff} = \frac{D^B_{mono} - D^B_{mix}}{D^B_{mono}}$$

D^{B}_{diff} = 0 means that species have the same relative abundance in mixture and in monoculture. D^{B}_{diff} < 0 means that the most abundant species in mixture are more phylogenetically distant from each other than are the most abundant species in monoculture.D^{B}_{diff} > 0 means that the most abundant species in mixture are more closely related than are the most abundant species in monoculture.

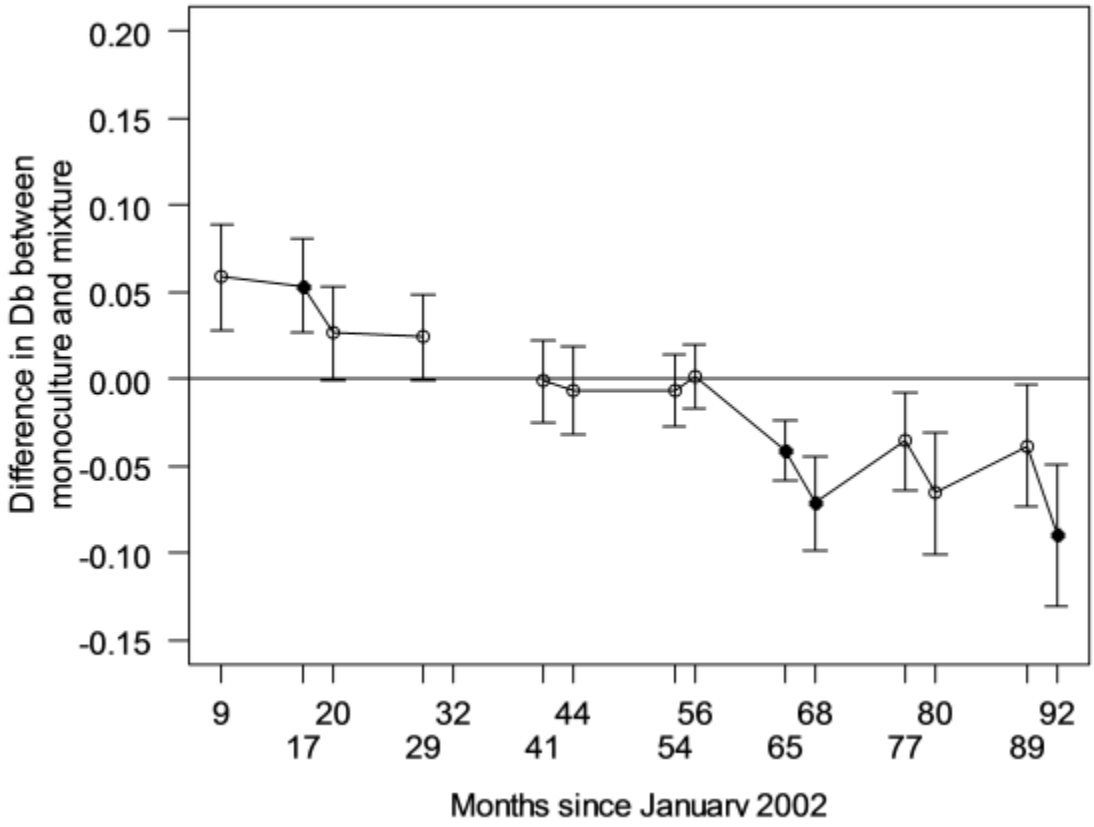

Fig. B1. Change in the difference in D^{B} between monocultures and mixtures (D^{B}_{diff}). Negative values of D^{B}_{diff} indicate that the most abundant species in mixture are less closely related to each other than are the most abundant species in monoculture. Positive values indicate the opposite: the dominant species in mixture are more closely related to each other than are the dominant species in monoculture.
The D^{B}_{diff} decreases with time. Values significantly different to 0 are shown with filled circles. Values at 9 months and 80 months are marginally significant (P = 0.06). Note that there are no values for time 32 months, summer 2004, because biomass was not sorted to species during this harvest.

Literature Cited

Hardy, O. J. 2008. Testing the spatial phylogenetic structure of local communities: statistical performances of different null models and test statistics on a locally neutral community. J. Ecol. 96:914–926.

Appendix C. One table and two figures presenting the correlation between sown species richness and sown phylogenetic diversity plus the minimal adequate models for the analysis of the fixed composition and reassembly experiments.

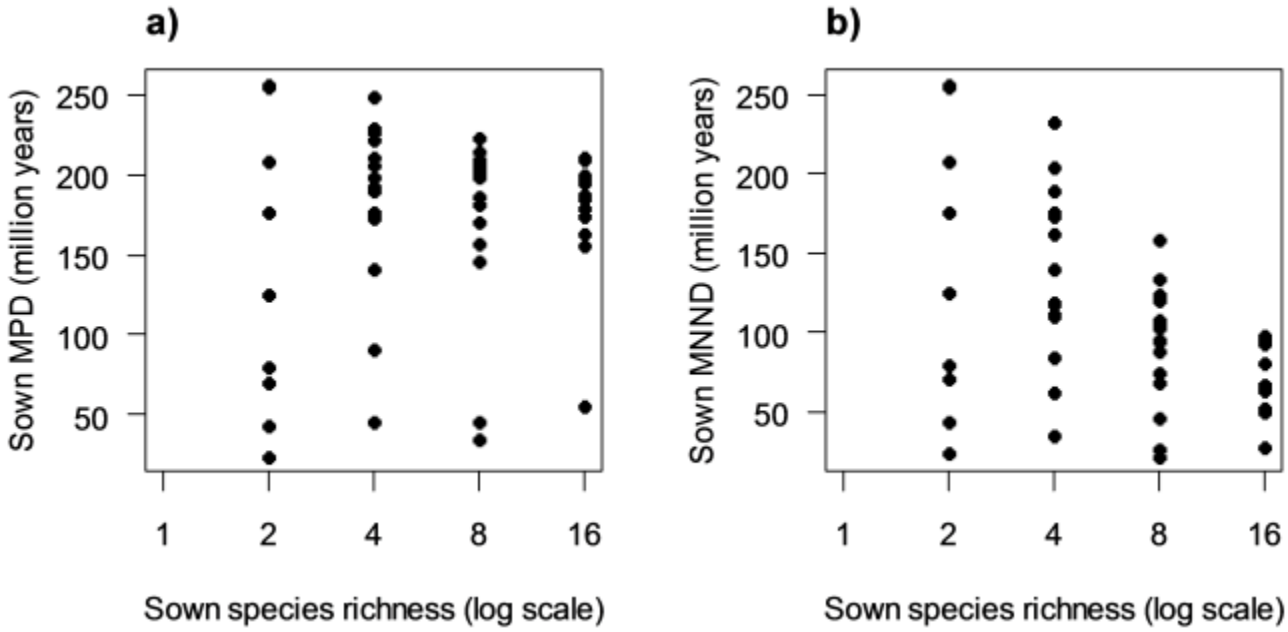

Fig. C1. The relationship between sown species richness and sown phylogenetic diversity. Sown species richness is uncorrelated with (a) Mean Pairwise Distance (MPD) ($P = 0.42$) but is correlated with (b) Mean Nearest Neighbor Distance (MNND) ($P < 0.001$). Neither measure of phylogenetic diversity can be calculated for monocultures. Note that for the main experiment only plots with 4, 8, or 16 species were included in the analysis. For the reassembly experiment all plots were included because species richness rapidly increased on the monoculture and two species mixture plots.

Table C1. The minimum adequate model for the analysis of APD values in the fixed composition experiment. Models were linear mixed-effects models with random effects for cover survey (time as a categorical factor with 15 time points) and plot (1–46). Time was fitted as a continuous variable, months since the start of the experiment, corresponding to a linear contrast within the random-effects term cover survey. Two measures of phylogenetic diversity, sown Mean Pairwise Distance (MPD) and sown Mean Nearest Neighbor Distance (MNND) were included. Sown richness was the sown species richness of the plot (4, 8, or 16 species). Functional group composition was represented as the presence absence of the four functional groups, legumes, grasses, small herbs and tall herbs. Estimates and standard errors come from the mixed model. MPD, MNND, and species richness were here scaled between 0 and 1 so that their parameter estimates are comparable. χ^2 and *P* values are for deletion of terms from this model. Terms which are marginal to higher order terms and therefore cannot be removed are indicated as marginal. Terms which were removed during model simplification are shown below, under terms removed; χ^2 and *P* values for deletion of these terms are shown.

Term	**Estimate**	**SE**	**χ^2**	***P***
Intercept	0.0717	0.0732	NA	
Time	-0.0018	0.0006	Marginal	
MPD	0.3251	0.1663	Marginal	
MNND	-0.0849	0.1216	Marginal	
Grasses	-0.0344	0.0396	Marginal	
Legumes	-0.1340	0.0386	Marginal	
Tall herbs	-0.0446	0.0555	Marginal	
Small herbs	-0.1438	0.0491	Marginal	
Sown richness	0.0991	0.0888	3.8	0.05
Time × MPD	-0.0054	0.0013	15.3	< 0.001
Time × MNND	0.0028	0.0008	10.5	< 0.01
Time × grasses	0.0007	0.0003	4.7	0.02
Time × legumes	0.0010	0.0003	9.3	< 0.01
Time × tall herbs	0.0012	0.0004	7.2	< 0.01
Time × small herbs	0.0023	0.0004	32	< 0.001
Terms removed				
Time × sown richness			0.11	0.73

Table C2. The minimum adequate model for the analysis of APD values in the reassembly experiment. Models were linear mixed effect models with random effects for cover survey (time as categorical factor with 6 time points) and plot (1–78). Time was fitted as a continuous variable, months since the start of the experiment, corresponding to a linear contrast within the random-effects term cover survey. Two measures of phylogenetic diversity, sown Mean Pairwise Distance (MPD) and sown Mean Nearest Neighbor Distance (MNND) were included; these are based on sown composition in 2002. Sown richness was the sown species richness of the plot (1, 2, 4, 8, or 16 species). Functional group composition was represented as the sown presence absence of the four functional groups, legumes, grasses, small herbs and tall herbs. Estimates and standard errors come from the mixed model. MPD, MNND, and species richness were here scaled between 0 and 1 so that their parameter estimates are comparable. χ^2 and *P* values are for deletion of terms from this model. Terms which are marginal to higher order terms and therefore cannot be removed are indicated as marginal. Terms removed during model simplification are shown below, under terms removed; χ^2 and *P* values for deletion of these terms are shown.

Term	**Estimate**	**SE**	**χ^2**	***P***
Intercept	-0.1198	0.0838	NA	
Time	0.0017	0.0015	Marginal	
Sown diversity	0.4244	0.0359	Marginal	
MPD	-0.0092	0.0010	Marginal	
MNND	0.0060	0.0009	Marginal	
Legumes	0.3405	0.0517	Marginal	
Time × sown diversity	-0.0064	0.0006	89	< 0.001
Time × MPD	0.0001	0.0000	37	< 0.001
Time × MNND	-0.0001	0.0000	22	< 0.001
Time × legumes	-0.0050	0.0009	32	< 0.001
Terms removed				
Small herbs			0.06	0.80
Tall herbs			0.69	0.40
Grasses			0.05	0.82
Time × grasses			3.0	0.08
Time × tall herbs			0.04	0.83
Time × small herbs			0.31	0.58

Appendix D. The effect of calculating APD in the reassembly experiment ignoring phylogenetic pattern in colonization.

Calculating APD ignoring phylogenetic pattern in colonization

The patterns in APD that we observed in the re-assembly experiment could be affected not only by shifts in abundance but also by shifts in composition. If interactions between dominant species, rather than the presence of evolutionarily distinct rare species, drive phylogenetic patterns then changes in the relative abundance of dominant species should be more important than compositional shifts in determining phylogenetic dispersion. To quantify this we recalculated APD ignoring any compositional shifts. We did this for the subplots opened to colonization by all 60 species, where only a subset of species successfully established. We calculated APD using two MPD values: (i) MPD between all species sown (i.e., all 60) and (ii) the MPD between all species that were found in the plot during a particular survey, i.e., those with cover > 0. If the species present in the community are overdispersed (or clustered) on the phylogeny of the species pool, i.e., if there is phylogenetic pattern in colonization, then APD calculated with these two different methods will differ: stronger evidence for phylogenetic dispersion will be found using (i) because (ii) ignores any phylogenetic pattern in composition. This therefore allowed us to test whether changes in APD on these plots were due to (i) composition and abundance shifts or (ii) only abundance shifts.

For the reassembly experiment, calculating APD ignoring phylogenetic pattern in colonization did not substantially change the results, although it increased the evidence for overdispersion, and reduced the evidence for initial clustering, as APD values tended to be lower when calculated ignoring phylogenetic pattern in composition (Fig. D1).

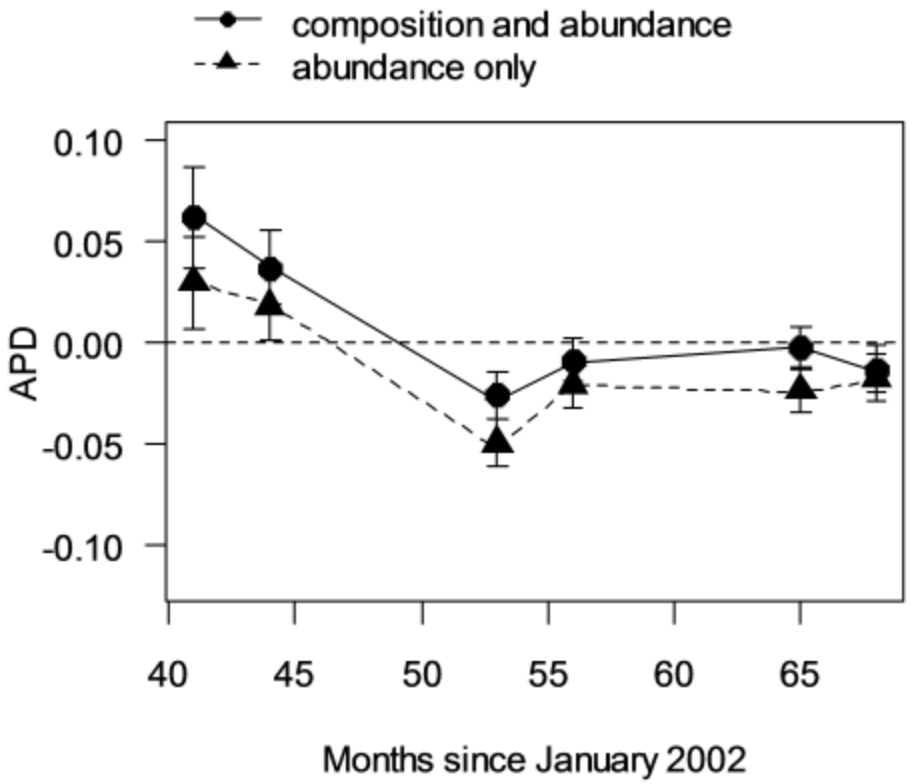

Fig. D1. Change in phylogenetic dispersion over time showing the effect of shifts in composition and abundance. For plots colonized by all 60 species in 2005, APD was calculated either taking into account abundance and composition (dots and solid lines) or ignoring phylogenetic pattern in composition caused by extinction/colonization (see above for more details).

Chapter 10

Non-linear patterns of relatedness in re-assembled grassland.

Fergus, A.J.F., Allan, E., Jenkins, T., Petermann, J. A., Roscher, C., Schmid, B., & Prinzing, A.; Manuscript.

Abstract

If closely related species share similar trait states, they should be less likely to co-occur. But if competitive exclusion of closely related species drives the divergence of trait states, then co-existence is possible. Neither of these ecological scenarios is evolutionary plausible. If environments sorted evolutionary lineages, they would never forsake an ancestral environment. If competition prevented co-existence of closely related species, then over time related species should occupy more and increasingly disparate environments than distantly related species do. Clearly this is not the case as neither of these evolutionary scenarios operates alone for any lineage, all lineages are influenced by both — they retain inherited environmental preferences and they explore novel environments. This raises the question, can biotic interactions, under the same regime of abiotic filtering, have opposing effects on the co-existence of related species? Alternatively, is relatedness and co-occurrence a non-linear relationship? In experimental grassland we followed the re-assembly of communities comprising a gradient of species (1, 2, 4, 8, 16, and 60 species) and functional group (1-4) richness, with varying species composition. Vascular plant communities were sown on a former arable field and for three years the richness treatments were maintained. We then added the entire species pool (60 species) to one plot in each community (a second remained as a control) and followed the re-assembly of communities without dispersal limitation for the subsequent five years. As was expected phylogenetic diversity converged with time. Mean pairwise distance between species increased, and mean nearest taxon distance decreased. The correlation between species co-occurrence and phylogenetic distance began negative, indicating phylogenetic conservatism, but this negative correlation consistently weakened with time. Examination of the trend in the relationship between co-occurrence (C*ij*) and phylogenetic distance (PD*ij*) revealed development of a ‘U’ shaped bimodal pattern. As communities re-assembled the co-occurrence of both closely related and distantly related species increased. By further breaking down co-existence patterns to lineage we reveal that the relationship between C*ij* and PD*ij* varied from negative, through neutral, to positive, for the four dominant families in the experiment. If separate lineages produce different levels of phylogenetic dispersion then the nature of species interactions within a lineage might vary between

lineages. This mechanism then directs biotic interactions within lineages and reinforces evidence that assembly of grassland communities is subject to non-random phylogenetic constraints.

Introduction

Evolutionary history has a strong imprint on community assembly. Darwin drew attention to the link between species belonging to the same genus and their similarities in habits, constitution and structure (Darwin 1859, Cahill *et al.* 2008, Proches *et al.* 2008, Thuiller *et al.* 2010). Analysis of ecological variation between closely related species supports Darwin's view, and regularly reveals such phenotypic and ecological similarity, also termed phylogenetic trait- or niche conservatism (Prinzing *et al.* 2001, Ackerly 2003, Losos 2008). These similarities between species promote the chances of closely related species successfully establishing together, as they are more likely to succeed in niches that resemble those to which both were formerly adapted (Ackerly 2003, Thuiller *et al.* 2010). This sorting process, controlled by local environmental conditions, is commonly referred to as the local or abiotic filter in community assembly literature. If abiotic filters were the only filtering processes influencing community assembly, local community compositions would converge under common abiotic conditions (Pfisterer *et al.* 2004, Fukami *et al.* 2005) and species belonging to the same genus would co-occur more often than expected at random.

Darwin, in what is popularly acknowledged as his naturalisation hypothesis, also declared that the competitive struggle between two species would be more intense if both species belonged to the same genus — due to aforementioned similarities their niches would overlap (Darwin 1859, Proches 2008). Diamond (1975) in articulating his assembly rules, developed these ideas and discussed permissible and forbidden combinations of coadjusted species based for the most part on competition for- and utilization of- resources. Interspecific plant competition is a major component of biotic filtering in the community assembly process, but the biotic filter also incorporates all other interactions between a plant and the living components of the community (Lawton 1987). Biotic filtering should lead to the exclusion of species sharing similar trait states via competitive exclusion (Gause 1934) limiting the similarity of species in a community

(Macarthur & Levins 1967). Therefore, given that closely related species tend to share similar trait states, it can be speculated that such species should co-occur less often (Webb *et al.* 2002 but see Cahill 2008). On the other hand, competitive exclusion could also drive the divergence of trait states of related species (character displacement) permitting closely related species to co-exist (Gause 1934, Brown & Wilson 1956, Prinzing *et al.* 2008).

Neither of these ecological scenarios is evolutionary plausible. If environments were sorting lineages, then evolutionary lineages should never leave the ancestral environment. If competition was preventing closely related species from co-existing, then closely related species should in the long run occupy a greater number of different environments than distantly related species. Any intermediate scenario would slow down the process but not change the evolutionary endpoint. Obviously, neither of these evolutionary scenarios operates alone for any lineage, all lineages are influenced by both — they retain inherited environmental preferences and they explore novel environments. This raises the question, can biotic interactions, under the same regime of abiotic filtering, have opposing effects on the co-existence of related species?

Community assembly may well be influenced by the phylogenetic proximity of community constituent species in multiple ways simultaneously. For example, phylogenetic proximity might affect abundant and rare species in different ways. Abiotic filtering of phylogenetically conserved niches should have a consistent influence on species regardless of their abundance, whereas biotic filtering — the interaction between closely related species — will primarily affect abundant species. Dominant or abundant species are increasingly likely to compete with a closely related species simply because their abundance dictates an increased chance that they will come into contact with a close relative. The interspecific competition component of the biotic filter is therefore density dependent. Similarly, the influence of species richness in a community might have a density dependant component.

Phylogenetic proximity may also have different effects depending on the time window analysed. Interactions between closely related species could either be synchronous direct interactions such as competition, or temporally offset mediated interactions involving an additional biotic player. Phylogenetic signal across mediated

interactions could indicate conservatism of host use by biotic go-betweens, such mediators include both above and belowground herbivores and pathogens, and mutualists like mycorrhizae and pollinators. The degree of host conservatism for each of these groups has been shown to vary (Vandenkoornhuyse *et al.* 2003, Agrawal 2007, Weiblen *et al.* 2006, Fontaine 2009, Futuyma & Agrawal 2009, Gossner *et al.* 2009) and there is some evidence that mutualists are increasingly generalists — conserved at higher taxonomic ranks — than pest groups that are commonly host specific (Weiblen *et al.* 2006). If some level of this dichotomy holds true, we would expect an increased likelihood that species belonging to the same family could co-occur, as they share mutualists. Conversely, species belonging to the same genus would be less likely to co-occur as they share pests, this is akin to apparent competition if increasing abundance of the first species is driving the effect. Mediated interactions, like direct competition, will also be density dependent, as pest or mutualist abundance will only be high enough to have spill over effects if their host plants are abundant enough in a community.

The effect of phylogenetic proximity on co-existence might also depend on the overall phylogenetic diversity in a community. With increasing species richness one would expect the mean distance between species to increase as there is on average more distance between each species. This increase would be automatic regardless of whether additional species joining a community were close or distant relatives to community occupants. However, how the distance between closest relatives responds to increasing species richness would be contingent on the regional species pool that additional species were being drawn from.

The effect of phylogenetic position on community assembly is usually considered to be linear, with proximity between species expected to increase or to decrease the probability of establishment (Cahill *et al.* 2008, Proches *et al.* 2008, Cadotte 2009, Thuiller *et al.* 2010). However, given the examples mentioned above we could expect the relationship might be non-linear. It is evident that an experiment is needed to test these effects of phylogenetic proximity on co-existence without confounding effects of abiotic habitat filtering. We followed the experimental re-assembly of controlled grassland communities with a gradient of species and functional diversity, and different species compositions. Communities were grown and their treatments maintained for

three years. We then added the entire species pool to each community and followed the re-assembly of communities without dispersal limitation for the subsequent five years. Using co-occurrence matrices we assessed the development of community phylogenetic patterns and related these to our expectations of abiotic and biotic filtering.

Methods

Experimental design

This study was conducted in the Jena Experiment (Germany), a large experimental platform designed to examine the effects of grassland biodiversity on ecosystem functioning (Roscher *et al.* 2004). The experimental site is on the floodplain of the Saale River near the city of Jena (Thuringia, Germany, 50°55' N, 11°35' E, 130 m a.s.l.). The mean annual air temperature is 9.3 °C, and the mean annual precipitation is 587 mm (Kluge and Müller-Westermeier 2000). The soil is a nutrient-rich Eutric Fluvisol developed from up to 2m-thick loamy fluvial sediments. Until the establishment of the biodiversity experiment the land was used for arable crops having been converted from grassland in the 1960s. The field was ploughed and kept fallow in 2001, and after being harrowed repeatedly, experimental grassland communities were sown in May 2002. Seventy-eight experimental plots were established with randomly assembled communities of 1, 2, 4, 8, or 16 species. The Jena Experiment species pool consisted of 60 native central European plant species chosen to resemble semi-natural species-rich mesophilic grassland, akin to a Molinio-Arrhenatheretea meadow (Ellenberg 1988). The 60 species were categorized into four functional groups derived from a cluster analysis of ecological and morphological traits, the groups were 16 grasses, 12 legumes, 12 small forbs, and 20 tall forbs. The number of functional groups (richness of functional groups) was varied as much as possible within the species richness levels to achieve an almost orthogonal design with respect to functional-group composition and species richness (Roscher *et al.* 2004). Communities were established in 20x20 m plots that were arranged randomly in four blocks. Each block also contained a bare ground plot (without vascular plants) and a plot sown with a mixture of the complete 60 species pool. The sown species combinations were maintained by weeding twice per year (April, July). Herbicides were used as spot-treatments against some weeds (*Cirsium arvense*

(L.) Scop., *Rumex* spec.), and where sown species combinations allowed for their application (bare ground plots, against herbs in pure grass communities and against grasses in pure herb communities, respectively). In addition, two succession plots (without sowing a seed mixture and alowing for spontaneous colonisation of vascular plants) were established. Plots were mown bi-annually and no fertiliser was applied.

Our experiment was nested within this larger experimental platform; within each 20x20 m plot we marked two 2x2.25 m subplots for a seed addition experiment. One of these subplots received a seed-addition treatment that included seeds of the complete 60 species pool while the other subplot was used as a control. In the seed addition subplot 1000 viable seeds/m2 (following standard laboratory tests) were added between the April 13 and 18 in 2005, the 1000 seeds were divided equally among the 60 species. A mixture containing seeds of all species of the experimental species pool at equal proportions was assembled for the seed addition treatment. Aimed sowing density amounted to 1000 viable seeds per m2. The usage of the same seed mixture for all plots irrespective of plot species number and composition ensured that each potential internal invader was sown with equal density in all plots, but reduced the number of sown invader seeds with increasing species
richness (e.g. 938 seeds out of 1000 seeds sown per m2 were potential invaders in monocultures, but only 733 out of 1000 were potential invaders in 16-species mixtures). Seed material was prepared following the same protocol as used during the establishment of the plots in 2002 including viability tests, pre-treatments of seeds and adjustment for germination rates. All subplots were sown in the period 13-18 April 2005. Seeds were mixed with groats of soya as bulking agent to guarantee an even distribution of seeds over the subplot area because of highly heterogeneous seed shapes and sizes. Groats of soya alone were distributed in subplots without seed addition treatment. The topsoil was scratched slightly; a border was placed around the subplots during sowing to avoid drift into neighboring subplots and after hand-sowing the surface was raked to ensure that no seed material get caught on the established vegetation. In the control subplot all species that were not part of the original design were removed by weeding, in the seed addition subplot all species not part of the 60 species pool were removed by weeding. Biannual weeding campaigns caused minimal

soil and vegetation disturbance, weeding was completed in early April at the beginning of the growing season, and again in July after the first mowing. Above ground plant biomass (taller than 3 cm) was harvested twice a year for three years after the start of our seed addition experiment, and harvests were timed to coincide with the standard agricultural harvest in central Europe (late May and August). A 20x50 cm area was randomly selected and harvested in each subplot, and biomass was sorted into species (an exception to this is the first harvest in 2005). After sorting biomass was dried and weighed. Cover was recorded twice a year for 5 years after the seed addition treatment was applied. Cover of all species was assessed by the same person in May and August each year over the complete area (2x2.25 m) for both subplots.

Phylogeny

We searched GENBANK for four gene sequences commonly used in building angiosperm phylogenies (*rbcl, matk, 5.8s* and *its2)* (Benson *et al.* 2005). For six species we used congeners when sequence data were not available (*Bellis rotundifolia* (Desf.) Boiss. & Reuter instead of *Bellis perennis* L., *Campanula latifolia* L. instead of *Campanula patula* L., *Geranium tuberosum* L. instead of *Geranium pratense* L., *Onobrychis montana* DC. instead of *Onobrychis viciifolia* Scop. and finally *Pimpinella saxifraga* L. instead of *Pimpinella major* L.). The gene sequences were individually aligned for each gene in the program MUSCLE, and then collated into one large alignment comprising a total of 4065 bp (Edgar 2004). Models of DNA substitution were tested for each gene sequence separately and selected based on AIC values, using MODELTEST (Posada & Cranwell 1998). Bayesian partitioned phylogenetic analyses were conducted in BEAST v1.4.8 with *Amborella trichopoda* and *Magnolia grandiflora* as outgroups (Drummond 2007). Four separate repeats were conducted, and allowed to "run" for 100 million generations. The burn-in was removed from each run and converged phylogenies were combined to produce a posterior distribution of trees.

Bayesian phylogenetic reconstructions were implemented in BEAST v.1.4.8 (Drummond 2007), using an uncorrelated lognormal relaxed molecular clock model with a Yule speciation tree prior, assuming a constant per lineage speciation rate. A General Time Reversible (GTR +I +Γ) model of DNA substitution was used, and analyses were

partitioned across the four genes to allow varying rates of molecular evolution. To account for the broad taxonomic diversity included, and the patchy sequence data, and to improve convergence, we imposed three constraints based on the angiosperm supertree (Bremer *et al.* 2009): constraint 1= Asterids, constraint 2= Rosids and constraint 3= Poales. We used *Amborella trichopoda* and *Magnolia grandiflora* as outgroups. As the trees were not calibrated, the mean substitution rate was set to 1.0, so fixing the rate of substitution of internal nodes at substitutions/site. The four separate runs were inspected in TRACER v.1.4 [32] in order to assess convergence and estimate the burn-in. Convergence was determined by visual examination of plots of each parameter and on estimates of effective sample size (ESS), where ESS > 200 indicates convergence [35]. After discarding the burn-in for each chain, and assessing that the trees had converged in the same phylogenetic space, the tree files were combined using TREEANNOTATOR v.1.4.8 [32] to produce the posterior distribution of 26940 trees. The maximum clade credibility tree of the posterior distribution of trees, that is the tree with the maximum product of posterior probabilities was used for analyses and is presented here (figure 1).

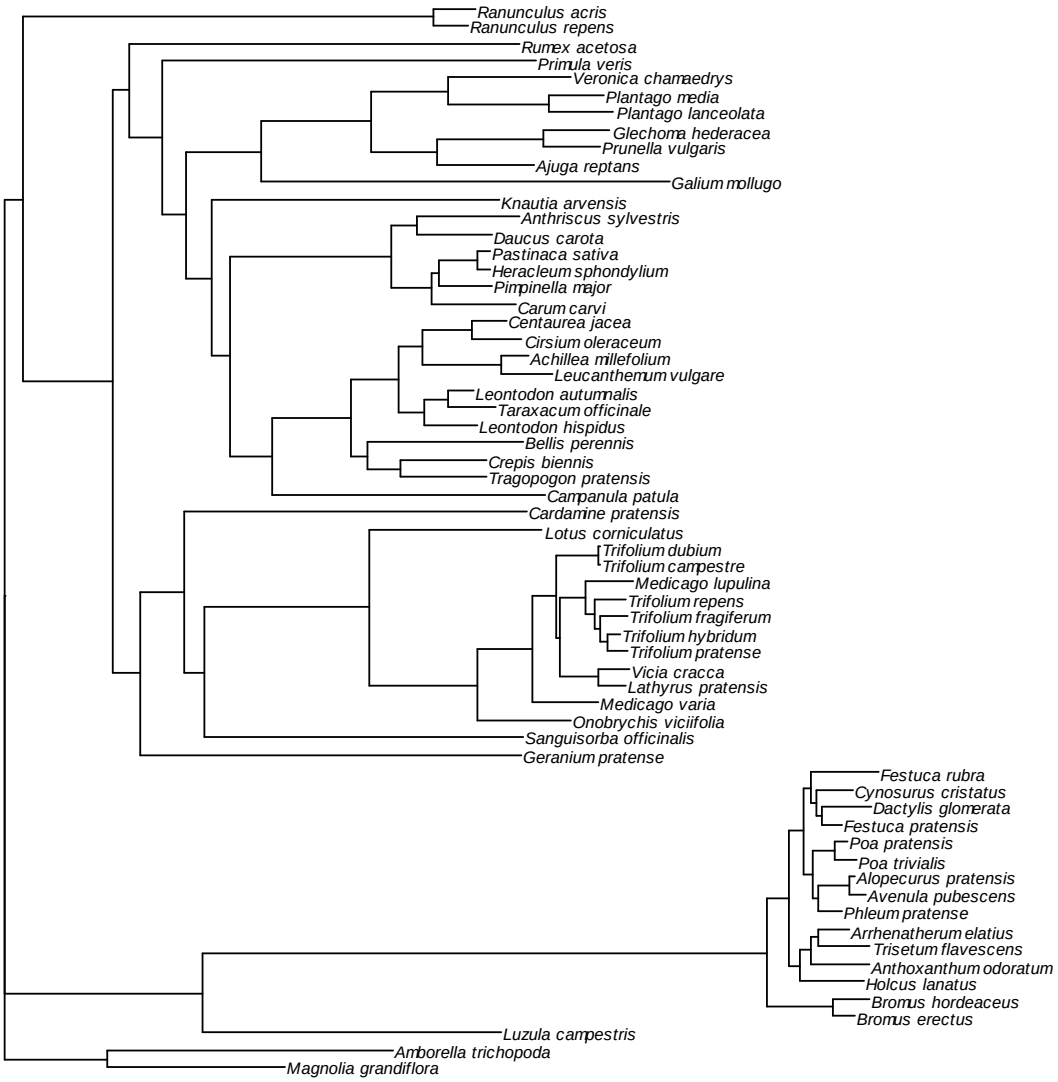

Figure 1. Maximum clade credibility phylogeny of the 60 species in the Jena Experiment species pool. Congeners were used for six species, see Methods for details.

Phylogenetic analysis

Phylogenetic distance (PD*ij*) between species was calculated using the cophenetic function in the R package 'ape' (Paradis et al. 2004). This function computes the pairwise distances between the pairs of tips from a phylogenetic tree using its branch lengths (Paradis et al. 2004). Phylogenetic distance is thus the sum of the estimated lengths of all intervening branches that connect two species on a phylogeny (Cavener-Bares et al. 2004). Faith's phylogenetic diversity is the sum of the total phylogenetic branch lengths connecting all species in a plot and was calculated using the pd function R package Picante (Kembel et al. 2010). Mean pairwise distance (MPD) between species was calculated using the ses.mpd function in the R package Picante, the function computes the standardized effect size of mean pairwise distances in communities (Webb et al. 2008, Kembel et al. 2010). The taxa labels null model was used when calculating MPD, this shuffles the PD*ij* matrix labels across all taxa included in distance matrix (Kembel et al. 2010). Similarly, mean nearest taxon distance (MNTD) between species was calculated using the ses.mntd function in the R package Picante, the function computes the standardized effect size of mean nearest taxon distances in communities (Webb et al. 2008, Kembel et al. 2010). The taxa labels null model was used when calculating MNTD, this shuffles the PD*ij* matrix labels across all taxa included in distance matrix (Kembel et al. 2010). MPD and MNTD values were calculated both with and without being weighted by abundance. MPD and MNTD values were calculated for each harvest and for each treatment.

A distance of co–occurrence was calculated using the species.dist function in the R package Picante, this function computes interspecific distances based on patterns of species co-occurrence in communities (Kembel et al. 2010). We opted to use the metric C*ij*, Schoener's index of co-occurrence, as this had previously been tested for similar analyses (Cavender-Bares et al. 2004, Hardy et al. 2008). Pairwise values of co-occurrence were calculated using a co-occurrence index (C*ij*) based on proportional similarity (Schoener 1970):

$$C_{ij} = 1 - 0.5 \sum_{k} \left| a_{ik} - a_{jk} \right|$$

Where *aik* is the abundance of species *i* in site *k* relative to the total abundance of species *i* over all sites (Hardy 2008). The comm.phylo.cor function in the R package Picante was used to calculate measures of community phylogenetic structure to patterns expected under a null model (Kembel et al. 2010). Community phylogenetic structure was measured as Pearson's correlation (*R*PD-CA) between the co-occurrence distance C*ij* and PD*ij*. A null model was used that shuffled phylogeny tip labels within the set of taxa present in community (Kembel et al. 2010). *R*PD-CA was compared to the respective averages obtained from the null model, lower or higher values of *R*PD-CA indicate spatial phylogenetic conservatism or overdispersion, respectively. Quantile regression slopes between C*ij* and PD*ij* were calculated using the comm.phylo.qr function in the Picante library in R. The function compares observed patterns to the patterns expected under a null model of species co-occurrence and phylogenetic distance, the taxa labels null model was used for comparison here (Kembel et al. 2010).

Additionally, a second correlation was calculated to obtain a measure of co-occurrence centred on 0 under independent species distributions. DO *ij* = (*P ij- Pi Pj*)/(*Pi Pj*), where *Pi*, *Pj* and *Pij* are the proportions of sites where species *i* occurs, species *j* occurs, and both species occur, respectively, this equates to a standardized difference between the observed and expected numbers of sites where both species occur (Hardy 2008). *R*PD-DO then is the correlation coefficient between PD*ij* and DO*ij*.

Additional statistical analysis

Change in phylogenetic diversity, MPD and MNTD values over time were analysed using linear mixed models, models were fit using the lme function in the R package nlme (Pinheiro et al. 2011). The analyses of phylogenetic diversity included the random effects block (1-4), plot (1-82) and a categorical random effect for harvest (1-10). The fixed effects included a continuous variable for time, measured as month after the experimental seed addition, a categorical fixed effect for sown species richness, a continuous variable for sown phylogenetic diversity (initial phylogenetic diversity), and a categorical fixed effect for treatment. The interactions: time x treatment, time x sown species richness, time x initial phylogenetic diversity, sown richness x treatment, and initial phylogenetic diversity x treatment were included in the model.

The separate analyses of MPD and MNTD included the random effects block (1-4), plot (1-82) and a categorical random effect for harvest (1-10). The fixed effects for these two models included a continuous variable for time, measured as month after the experimental seed addition, a categorical fixed effect for sown species richness, continuous variables each for MPD and MNTD at the first harvest, and a categorical fixed effect for treatment. The interactions: time x treatment, time x sown species richness, time x MPD or MNTD at harvest 1, sown richness x treatment, and MPD or MNTD at harvest 1 x treatment were included in the model.

Results

Phylogenetic diversity converged in the months following seed addition, in both plots with and without seed addition (table A1, $p>0.0001$, figure 2) suggesting that the weeding regime in the control plots was ineffective. The effect of the treatment was however significant, likely due to its effectiveness up until 2008 (table A1, $p>0.0001$). The significant interaction between months after seed addition and treatment alludes to this change. Species richness converged in a similar manner; this was reported by Peterman *et al.* (2010) from the first three years of biomass data from this experiment. Communities receiving seed addition converged at a higher level of PD than the control communities. The failure of the management regime to maintain the control plots from 2008 onwards, as seen in the convergence of species richness and PD, explains similar later responses in the phylogenetic metrics between treatment types.

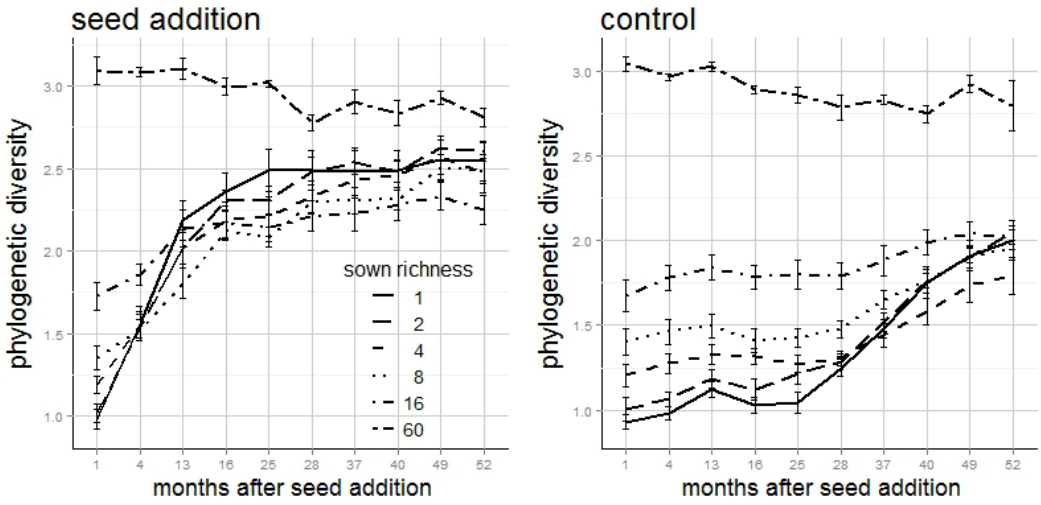

Figure 2. Patterns of convergence of phylogenetic diversity over time in plots with and without seed addition. Original sown species richness levels are plotted as separate lines.

Abundance weighted MPD increased consistently with time, with decreasing variance (table A3, p>0.0001, figure 3). In communities that received seed addition the average increase in MPD was higher, but not significantly. On the other hand, both the average abundance weighted MNTD and its variation decreased with time (table A5, p>0.0001, figure 3). Seed addition significantly influenced this decrease, communities receiving seed addition had on average a lower abundance weighted MNTD (table A5, p>0.0001, figure 4). Abundance weighted MPD also increased consistently with realised species richness (table A3, p>0. 0001, figure 4). A significant interaction with treatment (table A3, p=>0. 0001) reflects that species richness in the communities with seed addition was higher in the years before 2008. Abundance weighted MNTD decreased consistently with realised species richness (table A5, p>0.0001, figure 4). Similarly to MPD, this indicates that with increasing species richness there is a decrease in the distance to the nearest relative in a community. The significant effect of treatment (table A5, p=>0. 0001) is likely to reflect the faster increase in species richness in the control plots from 2008 onward.

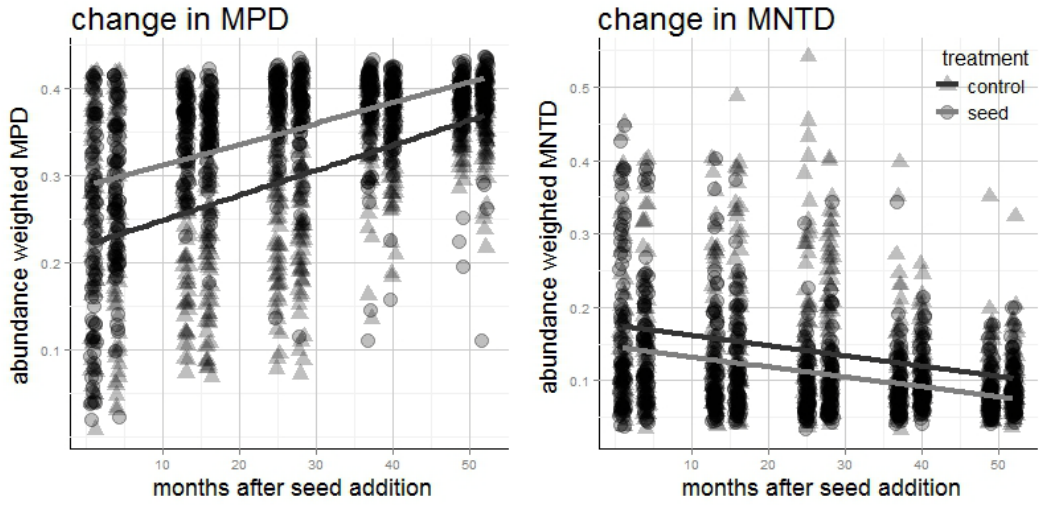

Figure 3. Change in abundance weighted MPD and abundance weighted MNTD with month after the seed sowing experiment began. Points are jittered to aid interpretation.

Pearson's correlation coefficient *R*PD-CA was initially negative in both control and seed addition plots (figure 5). This negative correlation is evidence for phylogenetic clustering in communities. In both control and seed addition plots negative correlations became increasingly positive with time, at the last harvests both plot types had a correlation of around 0, with trends in the data suggesting the increase in correlation would continue (figure 5). The slope of the increase was marginally higher in the control plots. The correlation for each harvest was significant, against a null model, up until 2008. A similar pattern was evident for Pearson's correlation coefficient *R*PD-DO, although a negative correlation remained in both plot types at the last harvests (figure 5). However the trend in the data indicates that with more time the correlation would become positive. There was a wider disparity between the *R*PD-DO coefficient in the control and seed addition plots (figure 5). The seed addition plots, up until the last two harvests, generally had a more positive correlation than the control plots.

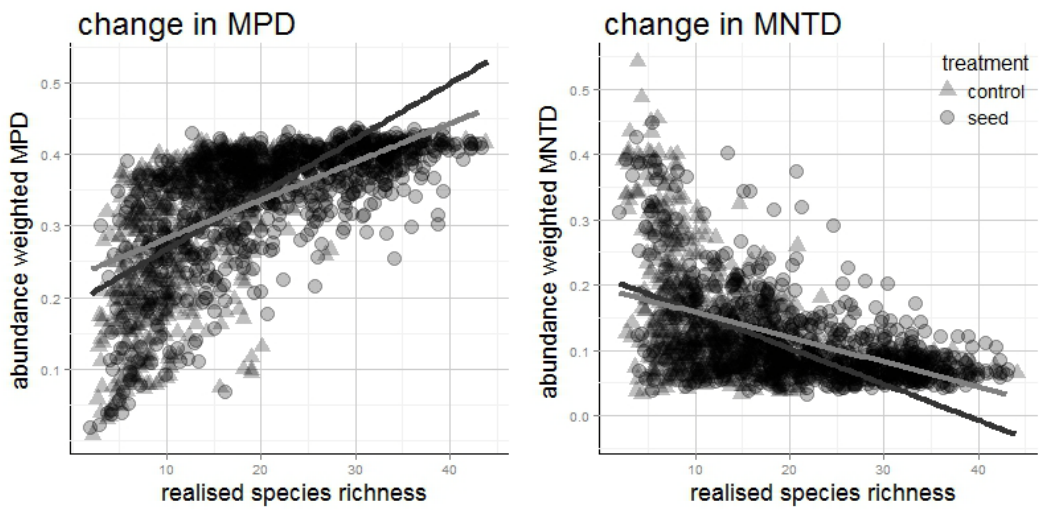

Figure 4. Change in abundance weighted MPD and abundance weighted MNTD with realised species richness. Points are jittered to aid clarity of pattern.

This correlation can be broken down by examining the pattern of C*ij* against PD*ij* over time. The pattern of C*ij* against PD*ij* from the May harvests with seed addition from 2005-2009 reveal a trend that becomes increasingly “U” shaped when a loess curve is fitted to the data (figure 6). Such a “U” shape indicates increasing co-occurrence of very closely and also distantly related species. A similar pattern was found in the control plots from the May harvest in each respective year (figure A1).

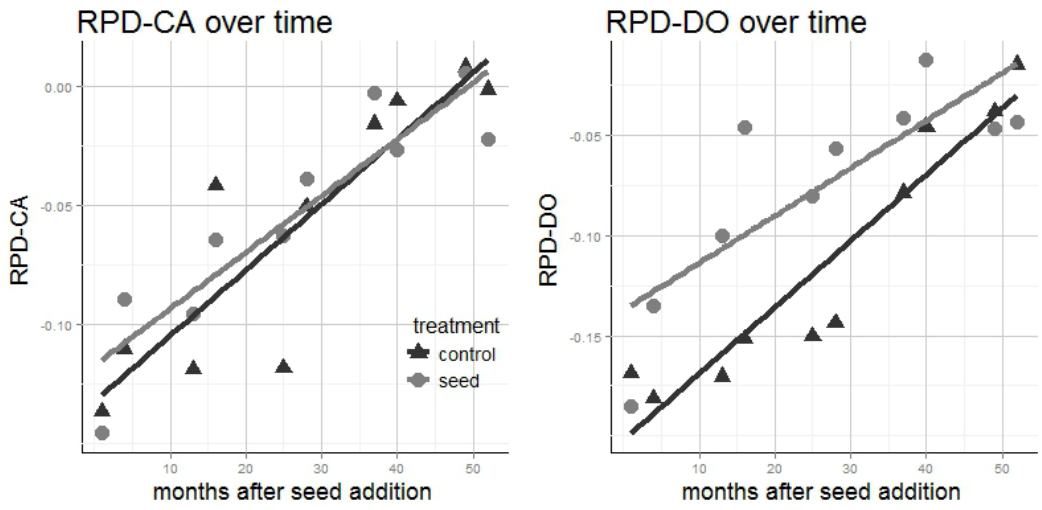

Figure 5. *R*PD-CA, the correlation between C*ij* and PD*ij*, analysed for each harvest and treatment. And *R*PD-DO, the correlation between DO*ij* and PD*ij*, analysed for each harvest and treatment.

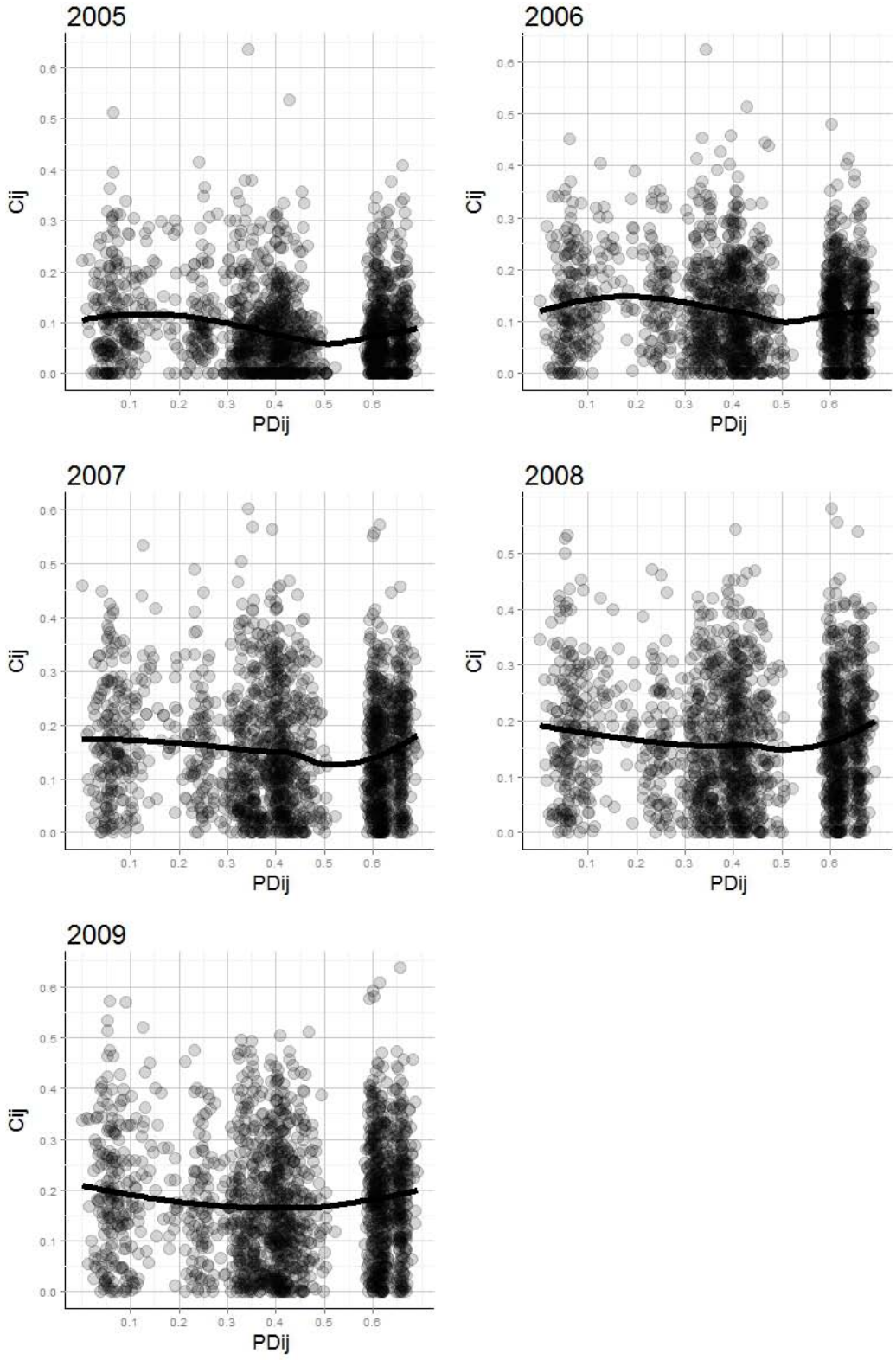

Figure 6. The change in the pattern of C*ij* against PD*ij* over time. All panels are from the May harvest that received seed addition in that respective year. Loess curves have been fitted.

The “U” shaped pattern of increasing co-occurrence of very closely and also distantly related species might indicate different patterns of co-occurrence within families of plants (figure 7). The four most abundant families in the experimental pool were Apiaceae (6 species), Asteraceae (10), Fabaceae (12 species) and Poaceae (15 species). We analysed each family separately for the May 2009 harvest, the last year of measurement and in early summer when the interaction intensity between species is highest. We explored the pattern of C*ij* against PD*ij* for each family by fitting a loess curve to the data (figure 7). To quantify these different patterns quantile regressions between C*ij* and PD*ij* were calculated and compared against a null model. The difference in patterns were not significantly different to null models, however slopes varied from negative, to neutral, to positive, depending on the family analysed. This result indicates that for different families there are different optimal distances of relatedness that promote co-occurrence (figure 7). Similar patterns were found for the control plots (figure 2A). It is worth considering how re-assembly influences the relationship in different lineages between C*ij* against PD*ij*. If we look at the May 2005 harvest and quantify the different patterns in each lineage with quantile regressions then again patterns were not significantly different to null models, however slopes for three families were in the opposite direction than 2009 (figure 8). C*ij* values in 2005 were generally much lower in 2005 than 2009, this suggests that for the two dominant lineages, Fabaceae and Poaceae, co-occurrence of closely related species within a lineage has increased with time (figure 8). Whereas for Apiaceae co-occurrence of more distantly related species within the lineage increases with time. The pattern remains indistinct for Asteraceae from one time window to another (figure 8).

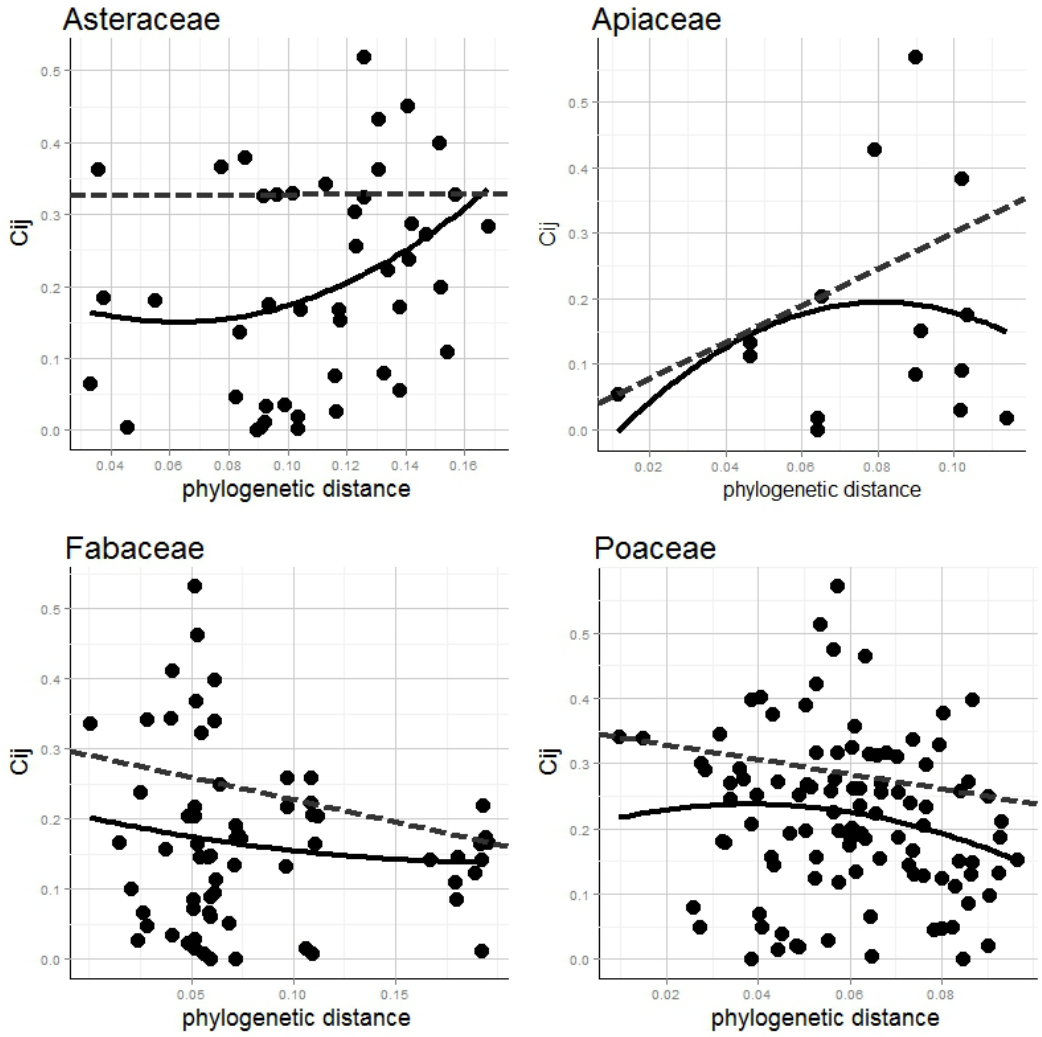

Figure 7: The change in the pattern of C*ij* against PD*ij* from the May 2009 harvest of plots with seed addition. Each panel, from top left clockwise, represent a single family: Asteraceae; Apiaceae; Fabaceae; and Poaceae, the four most diverse families in the experimental species pool. Loess curves have been fitted and quantile regression slopes have been plotted.

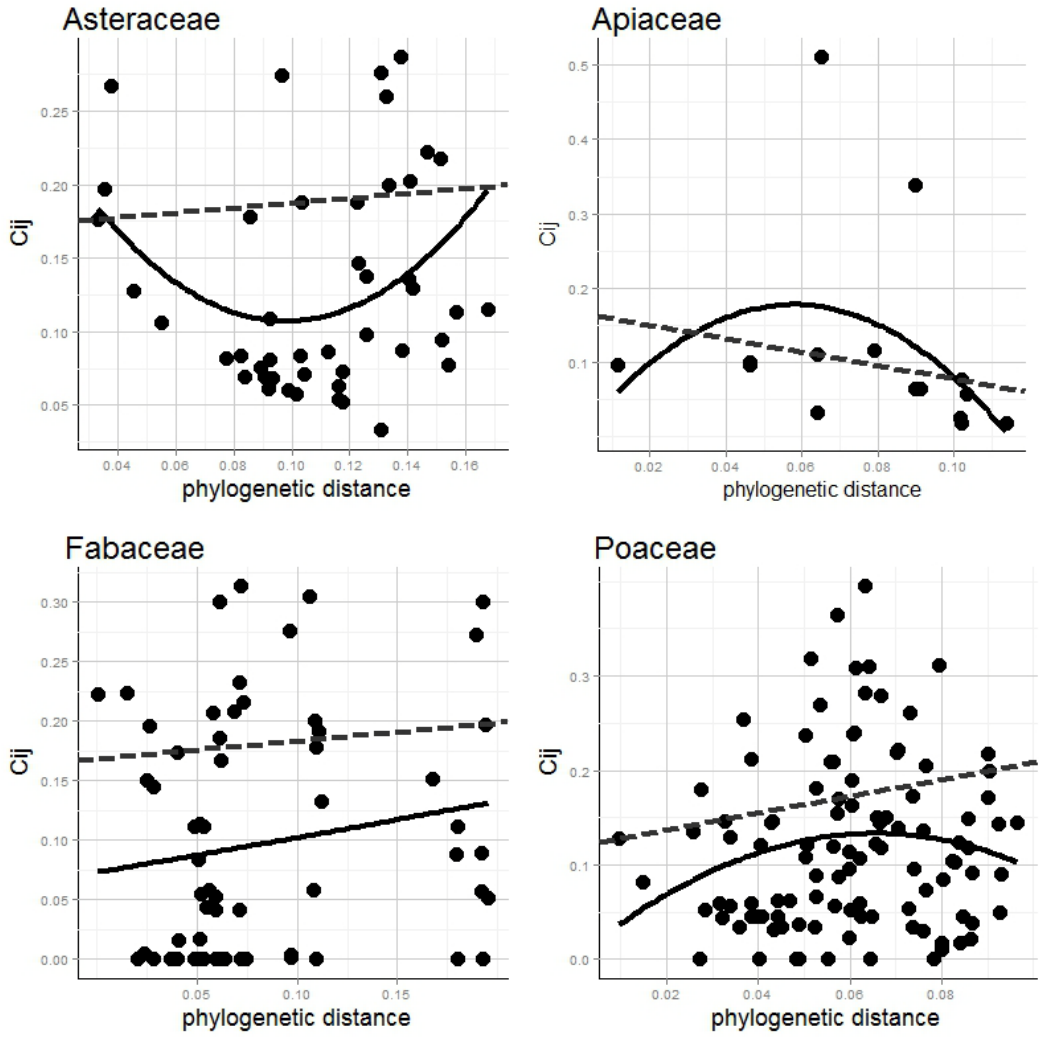

Figure 8: The change in the pattern of C*ij* against PD*ij* from the May 2005 harvest of plots with seed addition. Each panel, from top left clockwise, represent a single family: Asteraceae; Apiaceae; Fabaceae; and Poaceae, the four most diverse families in the experimental species pool. Loess curves have been fitted and quantile regression slopes have been plotted.

Discussion

The convergence of phylogenetic diversity across original sown species richness levels was expected in plots that received seed addition, but not in control plots. The result in control plots is most likely a result of ineffective maintenance of the species richness treatment. Generally this convergence supports a canon of experimental results demonstrating that increased species richness instils plant communities with higher

resistance to incoming or invading species (Tilman 1997, Naeem et al. 2000, Hector et al. 2001, Fargione et al. 2003, Pfisterer et al. 2004). High species richness generally equates to high phylogenetic diversity allowing comparison of these results to this literature. Increasing phylogenetic dispersion in plant communities has also been demonstrated to decrease how receptive a community is to alien species, the flipside of this is communities with low phylogenetic dispersion facilitate coexistence between native and alien species (Gerhold et al. 2011). In this experimental setting it has previously been demonstrated that establishment of incoming species is non-random (Petermann et al. 2010, Roscher et al. 2009a, Roscher et al. 2009 b). Established functional groups (a functional group was typically bound to a single family) of resident species negatively impact incoming species from the same functional group, resulting in complementary assembly, and community convergence in terms of species richness, functional group richness and evenness (Petermann et al. 2010). Convergence depends upon species richness, low diversity plant communities have been shown to be unstable and converge on higher richness levels (Roscher et al. 2009 a), whereas in more diverse communities increased utilisation of resources restricts incoming species (Roscher et al. 2009b).

The increase in abundance weighted MPD with time after seed addition could either be an outcome of the on-going addition of species into communities or the shuffling of abundance hierarchies within communities. Niche-based theory predicts incoming species to be different from those already present in a community; the addition of species from different lineages would increase the number of deeper splits in the phylogeny, increasing the mean phylogenetic distance between all pairs of species. However convergence in phylogenetic diversity and species richness (Petermann et al. 2010) had occurred within two years of seed addition, but the increase in MPD continued until the 5th year of measurement and potentially beyond. Examination of the trends in MPD based on co-occurrence alone (sans abundance data) reveals only a slight and less significant increase in MPD with time (figure A3, table A7). Inclusion of metrics of MPD weighted and not weighted by abundance is important as it permits a test for the presence of spatial phylogenetic structure and abundance phylogenetic structure (Helmus et al. 2007). One concern relates to testing for a spatial phylogenetic

structure against null models that randomize species abundances (Hardy 2008), as neutral processes have been shown to bring about complex patterns in the distribution of species abundance (Ulrich 2004). The trends in increase were similar for both the control and seed addition plots, abundance weighted MPD was generally higher for the latter, indicating a minor role for dispersal limitation, which slows the process but not its trajectory.

The decrease in abundance weighted MNTD with time indicates that the mean distance between each species and its closest relative is decreasing. This result, unlike that of MPD, remained consistent when the abundance of species was not included (figure A4). Without continuing increases in species richness and phylogenetic diversity this result suggests that a balance of incoming and outgoing species has been reached, and those now entering a community appear to succeed due to the presence of a close relative in a system. Cadotte and Strauss (2011) analysed a similar metric, mean nearest neighbour distance (MNND), over a shorter time, and found that in general species that were successful colonists were so in communities containing close relatives. The patterns of extinction from the communities studied revealed no phylogenetic signal in species extinction (Cadotte & Strauss 2011). In both control and seed addition plots MNTD decreased, the decrease was generally greater for plots with seed addition, suggesting dispersal limitation had some role in determining whether or not the incoming species was closely related to any of the resident species.

The increase in abundance weighted MPD was not reflected in the trend of MPD against realised species richness (figure A4). Therefore with increasing species richness species abundances are reshuffled resulting in an increase in the mean distance between pairs of species in a community. MNTD decreased with increasing species richness regardless of whether or not species abundance was incorporated. The implication is with increasing species richness there is a decrease in the distance to the nearest relative in a community. While this might be assumed to occur regardless, the extent of the effect is dependent upon the regional species pool. There are limits on our experimental species pool, 37 of our 60 species represent three families, yet this is arguably typical of grassland. But it could also be interpreted as driving some of the

MNTD result, as the dominance of these three families dictates the experimental species pool containing a high number of related species.

When we analysed the overall correlations (*R*PD-CA and *R*PD-DO) between co-ccurrence and phylogenetic distance across all communities per harvest we found a decreasing pattern of community phylogenetic clustering. When communities were allowed to re-assemble initial negative values of clustering gave way to positive values of *R*PD-CA, indicating overdispersion across all communities. The pattern of *R*PD-CA against PD over time (using the harvest in May each year) revealed increased lumping of close and distant related species increases with time, total *R*PD-CA positive values are most likely a result of greater lumping of distantly related species. A similar bimodal pattern of colonisation success was found by Cadotte and Strauss (2011) in a similar, but less diverse, experimental grassland. In that experiment, successful non-legume colonists were typically found co-occurring with close relatives. Whereas successful legume colonists occurred in plots that were colonized by distant relatives (Cadotte & Strauss 2011).

We explored whether or not the bimodal pattern of co-occurrence in our data set was in part a result of different patterns of co-occurrence within plant families. Differing directions of slopes for each of the four dominant families ran from negative, through neutral, to positive. This result indicates that for different families there are different optimal distances of relatedness that promote co-occurrence. These trends changed direction for three families with time; the re-assembly process then influences different lineages in different ways. Unlike Cadotte and Strauss (2011) we found within the family Fabaceae increased co-occurrence with increasing relatedness. The possibility that phylogenetic lineages might differ in the ways in which relatedness affects coexistence is a potentially important driver of assembly. If some lineages tend to move towards coexistence of increasingly closely related species, other lineages tend to move towards the opposite, with the even other lineages coexisting more at intermediate relatedness. This may demonstrate that different lineages produce different levels of phylogenetic dispersion which has multiple possible consequences for ecosystem functioning. There is good evidence suggesting that species evolve within communities as biotic interactions influence the evolutionary process (Neuhauser et al. 2003, Cavender-Bares

et al. 2006). If species within different lineages interact in different ways than those in other lineages then biotic interactions in plant communities depend on phylogenetic dispersion within the lineages in a community. In certain lineages, species may evolve in response to exposure to closely related species, this would impact competitive intensity (Webb *et al.* 2002), the role of shared natural enemies (Weiblen *et al.* 2006, Futuyma & Agrawal 2009, Gossner *et al.* 2009), shared mutualists (Vandenkoornhuyse *et al.* 2003, Fontaine 2009), and species which facilitate optimized abiotic variables. In other lineages species may be evolving in response to exposure to distantly related species, which would influence the factors mentioned above in potentially opposite or at least dissimilar ways. Responses to phylogenetic dispersion within a lineage then would not be due to any particularity of the respective environments that lineages occupy but it would reflect the assembly processes triggered by these lineages. This supports growing evidence that evolutionary processes, related to trait state dispersion, under niche conservation, influence assembly in plant communities (McPeek 1996, Webb et al. 2002, Ackerly 2003, Cavender-Bares et al. 2004, Wiens et al. 2010). The most important questions this research generates include what controls trait-state dispersion in a lineage? And is niche conservation consistent across different degrees of dispersion? This research provides further evidence that re-assembly of grassland communities is subject to non-random, non-linear phylogenetic constraints.

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Appendix A: Supporting tables and figures.

Table A1. Anova output for mixed effects model or phylogenetic diversity.

effect	nDF	dDF	F-value	p-value
(Intercept)	*1*	*812*	*5713.84*	*<.0001*
month after sown	*1*	*8*	*119.54*	*<.0001*
sown richness	*5*	*768*	*152.21*	*<.0001*
initial phylogenetic diversity (IPD)	*1*	*768*	*30.58*	*<.0001*
seed addition	*1*	*812*	*1585.73*	*<.0001*
month after sown x sown richness	*5*	*768*	*36.17*	*<.0001*
month after sown x IPD	*1*	*768*	*7.77*	*0.0055*
month after sown x seed addition	*1*	*812*	*49.73*	*<.0001*
sown richness x seed addition	*5*	*812*	*49.57*	*<.0001*
IPD x seed addition	*1*	*812*	*7.35*	*0.0069*

Table A2. Table of estimates for mixed effects model for phylogenetic diversity.

effect	value	SE	DF	t-value	p-value
(Intercept)	0.8526	0.0633	818	13.4769	0.0000
month after sown	0.0212	0.0020	8	10.5335	0.0000
sown richness 2	-0.0591	0.0543	768	-1.0876	0.2771
sown richness 4	-0.0488	0.0658	768	-0.7411	0.4589
sown richness 8	-0.0986	0.0816	768	-1.2085	0.2272
sown richness 16	0.0570	0.1161	768	0.4907	0.6237
sown richness 60	0.3012	0.3027	768	0.9953	0.3199
initial phylogenetic diversity (IPD)	0.4121	0.0773	768	5.3346	0.0000
seed addition	0.4376	0.0320	818	13.6600	0.0000
month after sown x sown richness 2	0.0014	0.0017	768	0.8348	0.4041
month after sown x sown richness 4	-0.0026	0.0021	768	-1.2299	0.2191
month after sown x sown richness 8	-0.0013	0.0026	768	-0.4850	0.6278
month after sown x sown richness 16	-0.0057	0.0037	768	-1.5365	0.1248
month after sown x sown richness 60	-0.0013	0.0096	768	-0.1339	0.8936
month after sown x IPD	-0.0068	0.0025	768	-2.7867	0.0055
month after sown x seed addition	0.0063	0.0010	818	6.1741	0.0000
sown richness 2 x seed addition	-0.0488	0.0501	812	-0.9745	0.3301
sown richness 4 x seed addition	-0.0480	0.0607	812	-0.7907	0.4293
sown richness 8 x seed addition	-0.2215	0.0753	812	-2.9432	0.0033
sown richness 16 x seed addition	-0.3345	0.1070	812	-3.1252	0.0018
sown richness 60 x seed addition	-0.0649	0.2790	812	-0.2328	0.8160
IPD x seed addition	-0.1930	0.0712	812	-2.7109	0.0069

Table A3. Anova output for mixed effects model for abundance weighted MPD.

effect	nDF	dDF	F-value	p-value
(Intercept)	*1*	*810*	*5725.53*	*<.0001*
month after sown	*1*	*8*	*110.01*	*<.0001*
sown richness	*5*	*770*	*69.87*	*<.0001*
MPD at harvest 1	*1*	*810*	*342.83*	*<.0001*
seed addition	*1*	*810*	*518.28*	*<.0001*
month after sown x sown richness	*5*	*770*	*40.68*	*<.0001*
month after sown x MPD at harvest 1	*1*	*810*	*404.43*	*<.0001*
month after sown x seed addition	*1*	*810*	*10.89*	*0.0010*
sown richness x seed addition	*5*	*810*	*19.22*	*<.0001*
MPD at harvest 1 x seed addition	*1*	*810*	*128.84*	*<.0001*

Table A4. Table of estimates for mixed effects model for abundance weighted MPD.

effect	value	SE	DF	t-value	p-value
(Intercept)	0.0130	0.0110	810	1.1871	0.2355
month after sown	0.0068	0.0003	8	20.2971	0.0000
sown richness 2	-0.0254	0.0092	770	-2.7617	0.0059
sown richness 4	-0.0009	0.0094	770	-0.0990	0.9212
sown richness 8	-0.0129	0.0099	770	-1.3039	0.1927
sown richness 16	-0.0034	0.0107	770	-0.3186	0.7501
sown richness 60	0.0177	0.0166	770	1.0680	0.2858
MPD at harvest 1	0.9048	0.0308	810	29.3482	0.0000
seed addition	0.1354	0.0077	810	17.5499	0.0000
month after sown x sown richness 2	0.0006	0.0003	770	2.1795	0.0296
month after sown x sown richness 4	0.0001	0.0003	770	0.5251	0.5996
month after sown x sown richness 8	0.0002	0.0003	770	0.7518	0.4524
month after sown x sown richness 16	0.0005	0.0003	770	1.6685	0.0956
month after sown x sown richness 60	0.0005	0.0005	770	1.0396	0.2988
month after sown x MPD at harvest 1	-0.0178	0.0009	810	-20.1090	0.0000
month after sown x seed addition	-0.0005	0.0001	810	-3.2998	0.0010
sown richness 2 x seed addition	0.0186	0.0079	810	2.3578	0.0186
sown richness 4 x seed addition	-0.0007	0.0081	810	-0.0840	0.9331
sown richness 8 x seed addition	0.0039	0.0085	810	0.4622	0.6440
sown richness 16 x seed addition	-0.0012	0.0092	810	-0.1284	0.8978
sown richness 60 x seed addition	0.0061	0.0143	810	0.4278	0.6689
MPD at harvest 1 x seed addition	-0.3040	0.0268	810	-11.3505	0.0000

Table A5. Anova output for mixed effects model for abundance weighted MNTD.

effect	nDF	dDF	F-value	p-value
(Intercept)	*1*	*810*	*3028.7274*	*<.0001*
month after sown	*1*	*8*	*108.1386*	*<.0001*
sown richness	*5*	*770*	*21.7254*	*<.0001*
MNTD at harvest 1	*1*	*810*	*681.3432*	*<.0001*
seed addition	*1*	*810*	*153.4203*	*<.0001*
month after sown x sown richness	*5*	*770*	*37.4837*	*<.0001*
month after sown x MNTD at harvest 1	*1*	*810*	*239.3701*	*<.0001*
month after sown x seed addition	1	810	0.4332	0.5106
sown richness x seed addition	*5*	*810*	*12.7310*	*<.0001*
MNTD at harvest 1 x seed addition	*1*	*810*	*111.2017*	*<.0001*

Table A6. Table of estimates for mixed effects model for abundance weighted MNTD.

effect	value	SE	DF	t-value	p-value
(Intercept)	0.0230	0.0102	810	2.2592	0.0241
month after sown	0.0004	0.0003	8	1.3744	0.2066
sown richness 2	0.0195	0.0090	770	2.1560	0.0314
sown richness 4	0.0061	0.0092	770	0.6623	0.5080
sown richness 8	0.0067	0.0095	770	0.7036	0.4819
sown richness 16	0.0020	0.0102	770	0.1964	0.8444
sown richness 60	-0.0070	0.0151	770	-0.4639	0.6428
MNTD at harvest 1	0.8985	0.0310	810	29.0153	0.0000
seed addition	0.0099	0.0089	810	1.1046	0.2697
month after sown x sown richness 2	-0.0002	0.0003	770	-0.6618	0.5083
month after sown x sown richness 4	0.0005	0.0003	770	1.8893	0.0592
month after sown x sown richness 8	0.0007	0.0003	770	2.5486	0.0110
month after sown x sown richness 16	0.0009	0.0003	770	3.2363	0.0013
month after sown x sown richness 60	0.0007	0.0004	770	1.5400	0.1240
month after sown x MNTD at harvest 1	-0.0135	0.0009	810	-15.4798	0.0000
month after sown x seed addition	0.0001	0.0001	810	0.6582	0.5106
sown richness 2 x seed addition	0.0021	0.0077	810	0.2698	0.7874
sown richness 4 x seed addition	-0.0001	0.0079	810	-0.0072	0.9943
sown richness 8 x seed addition	0.0098	0.0081	810	1.2055	0.2284
sown richness 16 x seed addition	0.0110	0.0087	810	1.2645	0.2064
sown richness 60 x seed addition	0.0067	0.0129	810	0.5183	0.6044
MNTD at harvest 1 x seed addition	-0.2842	0.0270	810	-10.5452	0.0000

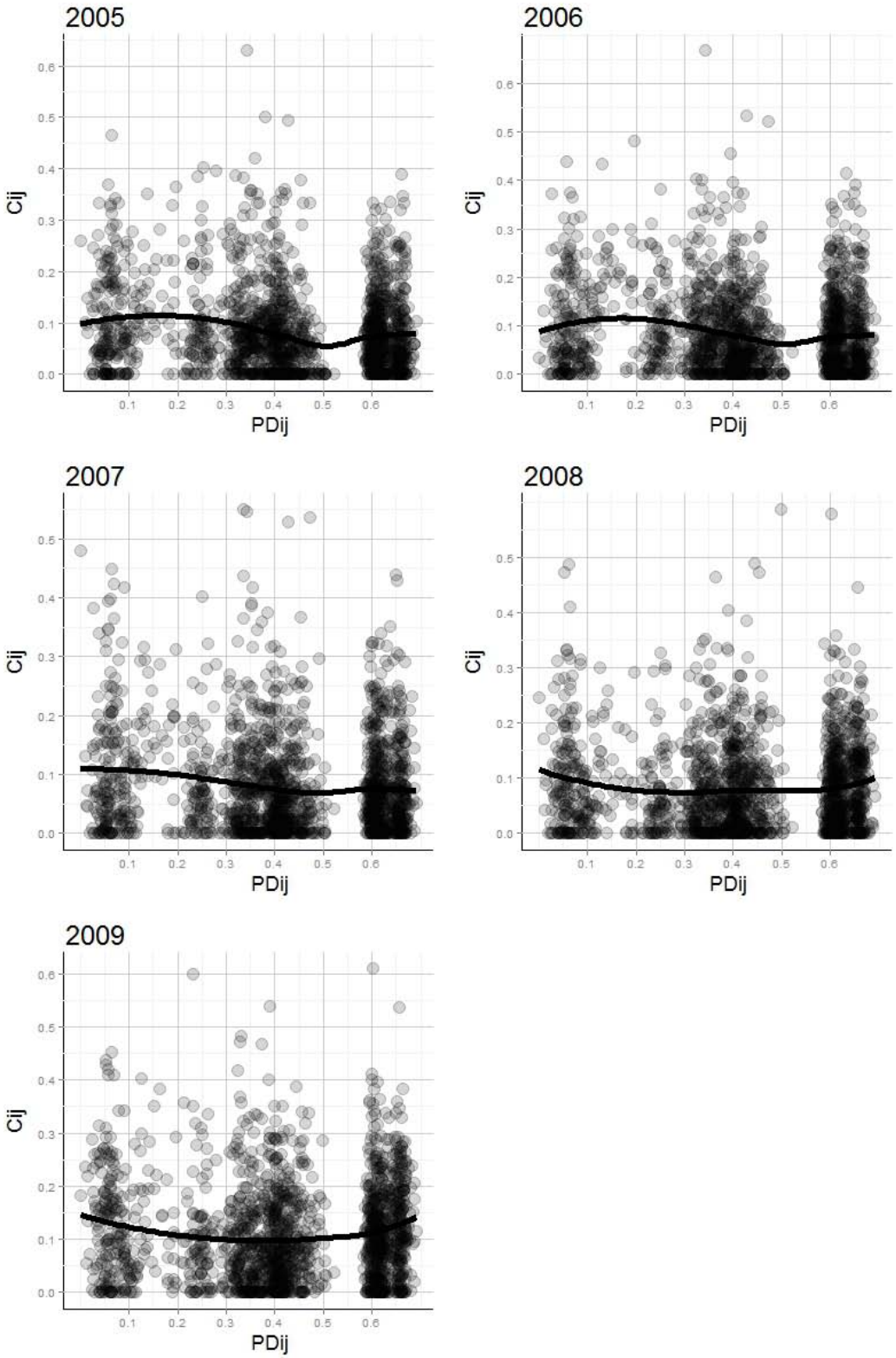

Figure A1. The change in the pattern of C*ij* against PD*ij* over time. All panels represent the control plots from the May harvest in that respective year. Loess curves have been fitted.

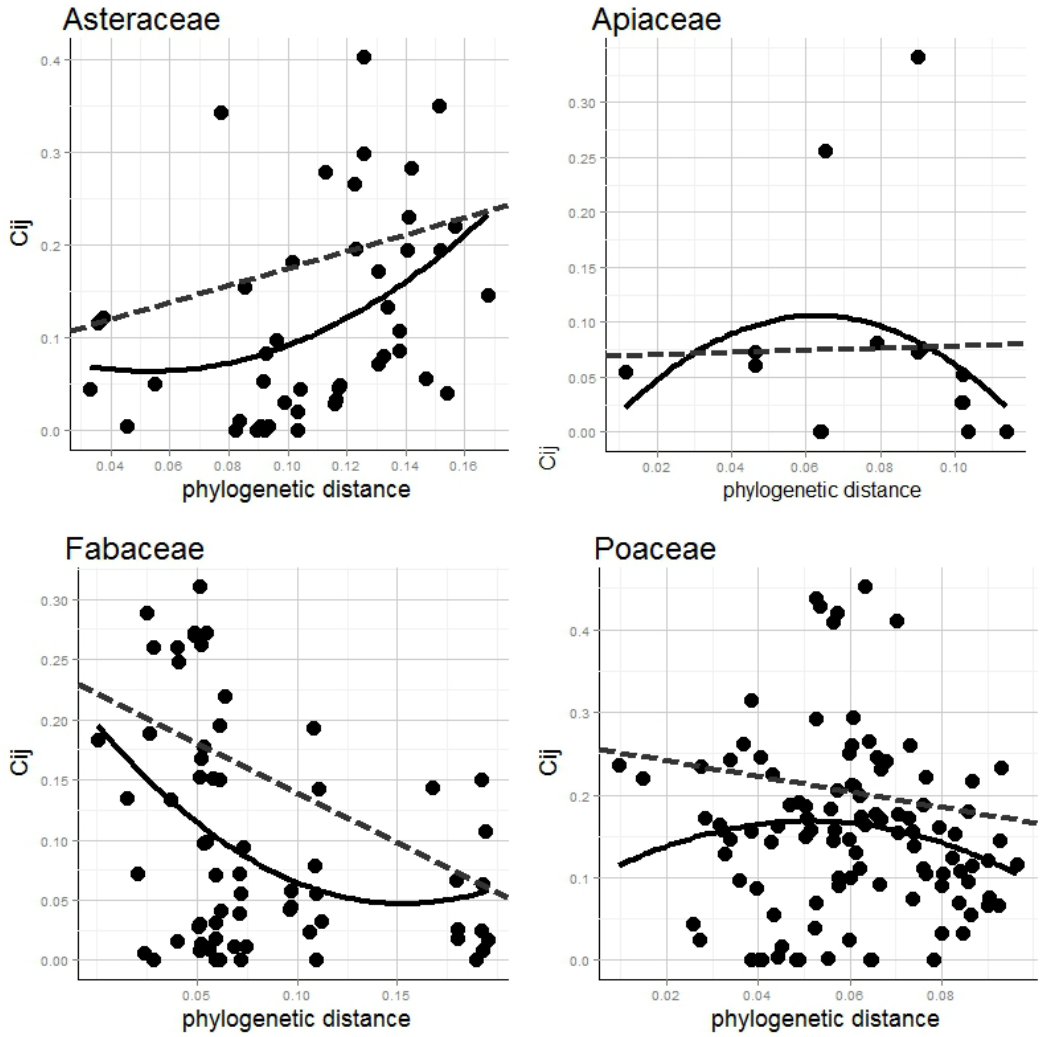

Figure A2. The change in the pattern of C*ij* against PD*ij* from the May 2009 harvest of control plots. Each panel, from top left clockwise, represent a single family: Asteraceae; Apiaceae; Fabaceae; and Poaceae, the four most diverse families in the experimental species pool. Loess curves have been fitted and quantile regression slopes have been plotted.

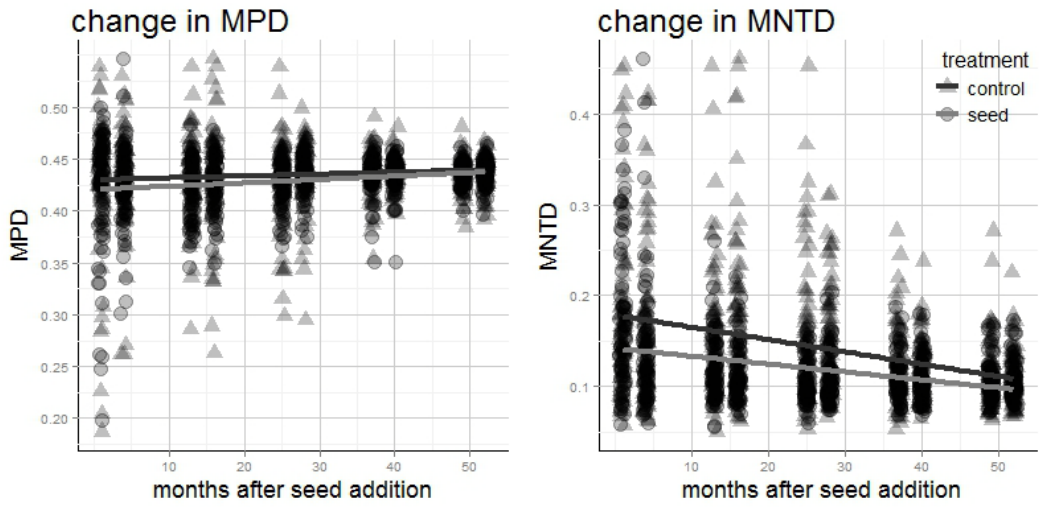

Figure A3. Change in MPD and MNTD with month after the seed sowing experiment began. Points are jittered to aid interpretation.

Table A7. Anova output for mixed effects model for MPD.

effect	nDF	dDF	F-value	p-value
(Intercept)	*1*	*810*	*231225*	*<.0001*
month after sown	*1*	*8*	*23.3300*	*0.0013*
sown richness	*5*	*770*	*9.5000*	*<.0001*
MPD at harvest 1	*1*	*810*	*418.5300*	*<.0001*
seed addition	*1*	*810*	*16.0400*	*0.0001*
month after sown x sown richness	*5*	*770*	*12.9900*	*<.0001*
month after sown x MPD at harvest 1	*1*	*810*	*368.6000*	*<.0001*
month after sown x seed addition	1	810	1.3600	0.2442
sown richness x seed addition	*5*	*810*	*9.1100*	*<.0001*
MPD at harvest 1 x seed addition	*1*	*810*	*53.7500*	*<.0001*

Table A8. Table of estimates for mixed effects model for abundance weighted MPD.

effect	value	SE	DF	t-value	p-value
(Intercept)	0.1605	0.0107	810	14.9773	0.0000
month after sown	0.0055	0.0003	8	17.7702	0.0000
sown richness 2	0.0095	0.0044	770	2.1704	0.0303
sown richness 4	-0.0005	0.0044	770	-0.1077	0.9143
sown richness 8	-0.0083	0.0044	770	-1.8750	0.0612
sown richness 16	-0.0093	0.0046	770	-2.0186	0.0439
sown richness 60	-0.0082	0.0069	770	-1.1856	0.2362
MPD at harvest 1	0.6361	0.0230	810	27.6985	0.0000
seed addition	0.0579	0.0094	810	6.1257	0.0000
month after sown x sown richness 2	-0.0001	0.0001	770	-0.4880	0.6257
month after sown x sown richness 4	0.0001	0.0001	770	0.7998	0.4241
month after sown x sown richness 8	0.0003	0.0001	770	2.2744	0.0232
month after sown x sown richness 16	0.0002	0.0001	770	1.4009	0.1616
month after sown x sown richness 60	0.0002	0.0002	770	0.9300	0.3527
month after sown x MPD at harvest 1	-0.0128	0.0007	810	-19.1177	0.0000
month after sown x seed addition	0.0001	0.0001	810	1.1653	0.2442
sown richness 2 x seed addition	-0.0120	0.0037	810	-3.2581	0.0012
sown richness 4 x seed addition	-0.0013	0.0037	810	-0.3487	0.7274
sown richness 8 x seed addition	0.0010	0.0037	810	0.2628	0.7928
sown richness 16 x seed addition	0.0060	0.0039	810	1.5573	0.1198
sown richness 60 x seed addition	0.0046	0.0058	810	0.7955	0.4266
MPD at harvest 1 x seed addition	-0.1498	0.0204	810	-7.3314	0.0000

Table A9. Anova output for mixed effects model for MNTD.

effect	nDF	dDF	F-value	p-value
(Intercept)	*1*	*810*	*3386.4070*	*<.0001*
month after sown	*1*	*8*	*70.9270*	*<.0001*
sown richness	*5*	*770*	*23.6640*	*<.0001*
MNTD at harvest 1	*1*	*810*	*655.1830*	*<.0001*
seed addition	*1*	*810*	*136.9610*	*<.0001*
month after sown x sown richness	*5*	*770*	*39.8280*	*<.0001*
month after sown x MNTD at harvest 1	*1*	*810*	*295.5020*	*<.0001*
month after sown x seed addition	*1*	*810*	*14.4440*	*0.0002*
sown richness x seed addition	*5*	*810*	*10.9550*	*<.0001*
MNTD at harvest 1 x seed addition	*1*	*810*	*35.1520*	*<.0001*

Table A10. Table of estimates for mixed effects model for MNTD.

effect	value	SE	DF	t-value	p-value
(Intercept)	0.0454	0.0088	810	5.1762	0.0000
month after sown	0.0007	0.0003	8	2.9049	0.0197
sown richness 2	0.0141	0.0067	770	2.0982	0.0362
sown richness 4	-0.0046	0.0068	770	-0.6797	0.4969
sown richness 8	-0.0031	0.0071	770	-0.4411	0.6593
sown richness 16	-0.0029	0.0076	770	-0.3803	0.7038
sown richness 60	-0.0191	0.0113	770	-1.6876	0.0919
MNTD at harvest 1	0.7572	0.0270	810	28.0266	0.0000
seed addition	-0.0098	0.0079	810	-1.2338	0.2176
month after sown x sown richness 2	0.0000	0.0002	770	-0.2252	0.8219
month after sown x sown richness 4	0.0004	0.0002	770	1.9331	0.0536
month after sown x sown richness 8	0.0005	0.0002	770	2.5054	0.0124
month after sown x sown richness 16	0.0005	0.0002	770	2.5956	0.0096
month after sown x sown richness 60	0.0006	0.0003	770	1.9518	0.0513
month after sown x MNTD at harvest 1	-0.0135	0.0008	810	-17.0708	0.0000
month after sown x seed addition	0.0004	0.0001	810	3.8006	0.0002
sown richness 2 x seed addition	-0.0098	0.0062	810	-1.5888	0.1125
sown richness 4 x seed addition	0.0089	0.0062	810	1.4364	0.1513
sown richness 8 x seed addition	0.0109	0.0064	810	1.6973	0.0900
sown richness 16 x seed addition	0.0091	0.0069	810	1.3177	0.1880
sown richness 60 x seed addition	0.0141	0.0104	810	1.3596	0.1743
MNTD at harvest 1 x seed addition	-0.1625	0.0274	810	-5.9289	0.0000

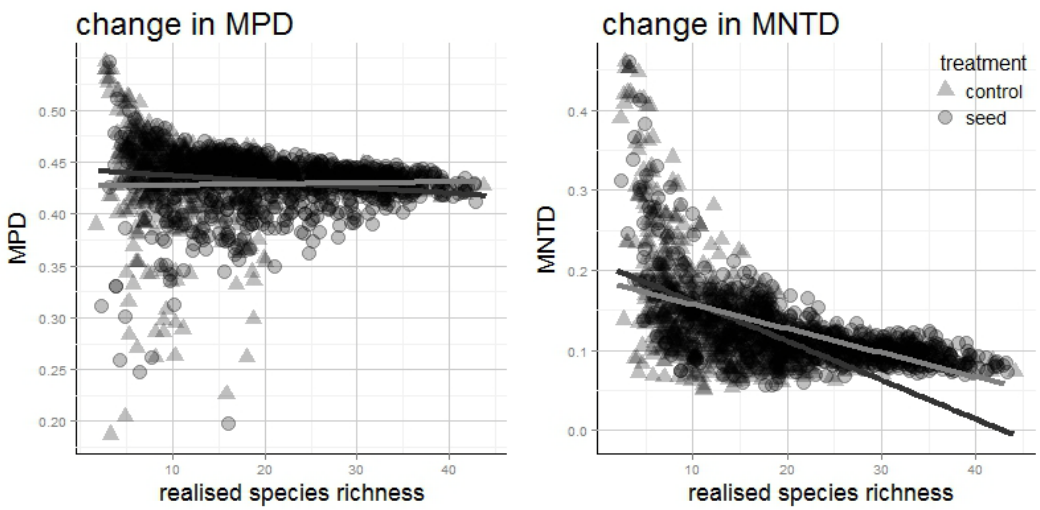

Figure A4. Change in MPD and MNTD with realized species richness. Points are jittered to aid clarity of pattern.

Chapter 11

Phylogenetically poor plant communities receive more alien species, which more easily coexist with natives

Gerhold, P., Pärtel, M., Tackenberg, O., Hennekens, S.M., Bartish, I., Schaminée, J.H.J, Fergus, A.J.F., Ozinga, W.A. & Prinzing, A. 2011. *The American Naturalist* 177: 668-680.

Abstract

Alien species can be a major threat to ecological communities, but we do not know why some community types allow the entry of many more alien species than do others. Here, for the first time, we suggest that evolutionary diversity inherent to the constituent species of a community may determine its present receptiveness to alien species. Using recent large databases from observational studies, we find robust evidence that assemblage of plant community types from few phylogenetic lineages (in plots without aliens) corresponds to higher receptiveness to aliens. Establishment of aliens in phylogenetically poor communities corresponds to increased phylogenetic dispersion of recipient communities and to coexistence with rather than replacement of natives. This coexistence between natives and distantly related aliens in recipient communities of low phylogenetic dispersion may reflect patterns of trait assembly. In communities without aliens, low phylogenetic dispersion corresponds to increased dispersion of most traits, and establishment of aliens corresponds to increased trait concentration. We conclude that if quantified across the tree of life, high biodiversity correlates with decreasing receptiveness to aliens. Low phylogenetic biodiversity, in contrast, facilitates coexistence between natives and aliens even if they share similar trait states.

Introduction

Alien species establish unequally across a given region; some ecological communities harbor many more alien species than do others (Chytrý et al. 2009). Moreover, aliens, once established in a community, can potentially cause extinctions of native species, alter relationships between species, and disturb nutrient cycling in food chains, in turn adversely impacting natural ecosystems worldwide (Vitousek et al. 1997; Blumenthal 2005; Chytrý et al. 2009). Understanding the reasons why some communities are more receptive to aliens and why aliens may cause extinctions in some communities and not in others would improve our ability to control future invasions and at the same time permit testing of major ecological theories (Blumenthal 2005; Callaway and Maron 2006). Community receptiveness to aliens has been associated with multiple biotic and abiotic factors (Maron and Connors 1996; Marler et al. 1999; Davis et al. 2000; Parker et al. 2006). Arguably the most persistent debate in invasion ecology is the role that

species richness plays in determining the receptiveness of a community to aliens. It has been suggested since Elton (1958) that communities rich in native species are less receptive to aliens because of increased competition and a lack of empty niches (Kennedy et al. 2002). However, the opposite has also been found (Stohlgren 1999; Levine 2000; Gilbert and Lechowicz 2005).

Studies that relate biodiversity to community receptiveness to aliens may have come to different conclusions because all species have been treated as evolutionary equals, but they are not. Some species are more closely related to each other, while others are more distantly related. Likewise, some communities are characterized by closely related incumbent native species, and others are characterized by distantly related incumbent species. This point has never been taken into account, despite the fact that Darwin (1859; Ludsin and Wolfe 2001) discerned an evolutionary dimension to patterns of receptiveness to aliens at a biogeographical scale. Darwin (1859, p. 106) found that the endemic biotas of oceanic islands were more vulnerable to aliens and explained this as a result of the evolutionary history of the island floras and faunas: “On a small island, the race for life will have been less severe.” Despite recent studies addressing whether the phylogenetic relationships among alien species (Cadotte et al. 2009) or among alien and native species (Ricciardi and Mottiar 2006; Proches et al. 2008) predict invasion success, phylogenetic relationships in the recipient community as a predictor of community receptiveness to aliens are unstudied.

The establishment of aliens in a community might be favored in either of two ways: aliens might easily replace natives, or aliens might easily coexist with natives. Both of these mechanisms might be related to the phylogenetic relatedness of the natives in the recipient community. First, replacement of natives by aliens might be favored if the recipient community is composed of closely related species, that is, if it has a low phylogenetic dispersion. Natives in such communities have generally been exposed only to closely related species and might therefore be naive to alien species from distantly related lineages, that is, to their specific competition pressures and their associated pests and pathogens. Natives might thus be inferior to and consequently replaced by distantly related aliens (replacement hypothesis; fig. 1A). For instance, island biotas have been considered naive to the numerous distantly related continental

lineages and therefore more vulnerable to replacement by aliens arriving from the continent (Darwin 1859). As in the island case, the species pools of particular types of environments were relatively closed to immigration from most lineages during the evolutionary past, despite dramatic transformations and redistributions, particularly during the past few thousand years (for remarkable examples, see Ortega et al. 1997; Prinzing et al. 2001; Crisp et al. 2009). Species from these isolated pools might have had no contact with and in turn lacked adaptation to antagonist species from a wide range of lineages. The local communities sampled today from these presumably naive species pools are phylogenetically underdispersed. Present-day processes such as dispersal limitation might further reduce phylogenetic dispersion of particular local communities. Incumbent populations are thus naive to competition with and the pathogens of distant lineages. Second, it might also be coexistence of aliens with natives that is favored in phylogenetically underdispersed recipient communities. In these communities, many phylogenetically distant lineages are absent, and aliens have a higher chance of belonging to such distant lineages. Distantly related species might then coexist more easily than closely related species (coexistence hypothesis; see below for mechanisms; fig. 1B; Webb et al. 2002; Prinzing et al. 2008). Both the replacement hypothesis and the coexistence hypothesis hence predict more aliens to establish in phylogenetically underdispersed communities than in overdispersed communities. Both hypotheses also predict the aliens in phylogenetically underdispersed communities to come from more distantly related lineages. These aliens will increase the phylogenetic dispersion of underdispersed recipient communities more than in overdispersed communities, where many lineages are already represented and thus an increase of dispersion is inevitably more difficult to achieve. However, the replacement hypothesis predicts that aliens entering phylogenetically underdispersed communities reduce native species richness, whereas the coexistence hypothesis predicts no such reduction.

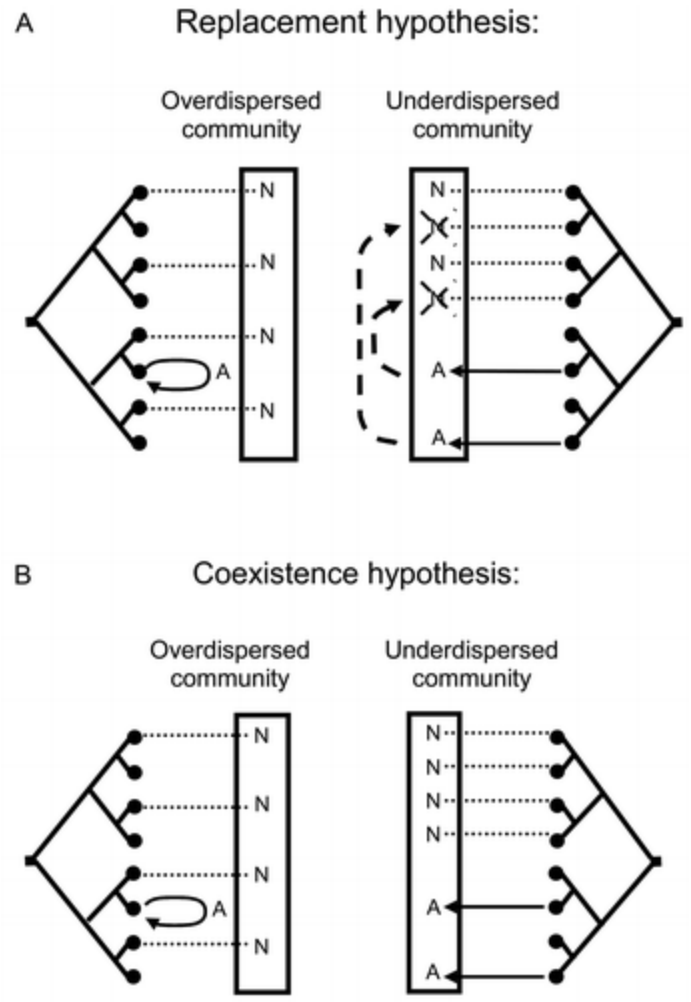

Figure 1. Hypotheses on the relationship between phylogenetic dispersion of local communities and how receptive they are to alien species. Dotted lines indicate the position of native species (N) in the phylogeny. Solid arrows indicate establishment (or not) of alien species (A) in a local community. Dashed arrows and crosses indicate replacement of native species by alien species. Hypothesis A, replacement of native species by aliens in phylogenetically underdispersed recipient communities. Hypothesis B, coexistence of native species and aliens in phylogenetically underdispersed recipient communities.

Coexistence between aliens and natives might be favored in the following two ways: (i) aliens could occupy different ecological niches or functional trait states than could natives or (ii) aliens and natives could better partition similar ecological niches or functional trait states than could natives among each other. Both coexistence mechanisms might prevail in communities of low phylogenetic dispersion. First, if fewer lineages represent fewer niche/trait states (as suggested in Webb et al. 2002), alien

species from distant lineages would likely represent trait states different from those of established natives and could thus coexist with natives. Second, in contrast, if coexisting species from the same lineage are under pressure to be particularly different in traits and niches (e.g., because they are particularly similar in the fundamental physiological strategies and the natural enemies they share; Gilbert and Webb 2007; Cavender-Bares et al. 2009), then communities assembled from fewer native lineages should represent highly divergent niche/trait states (for a review of mechanisms and confirmation of the pattern for most traits, see Prinzing et al. 2008). Alien species, being from distant lineages and thus unlikely to be similar to natives in ecological dimensions such as natural enemies, would not be under pressure to be different from the nonrelated natives. They could coexist with natives even if particular ecological or functional traits are similar to those of natives. Moreover, given the already high trait-state dispersion in the absence of aliens, aliens are likely to have traits similar to those of natives already present. The first hypothesis would thus predict that in communities without aliens, low phylogenetic dispersion correlates with low trait dispersion (e.g., low trait-state standard deviation for a continuous trait). The first hypothesis would also predict aliens to increase the dispersion of trait/niche states in phylogenetically underdispersed communities compared to overdispersed communities (fig. 2a). The second hypothesis, on the contrary, predicts that in communities without aliens, low phylogenetic dispersion correlates with high trait dispersion. Aliens would then add new species but not new trait states, the concentration of trait states would go up, and trait-state dispersion would decline (fig. 2b). We define these two hypotheses as coexistence with increasing trait-state dispersion and coexistence with increasing trait-state concentration. Note that different hypotheses might be true for different traits (Prinzing et al. 2008).

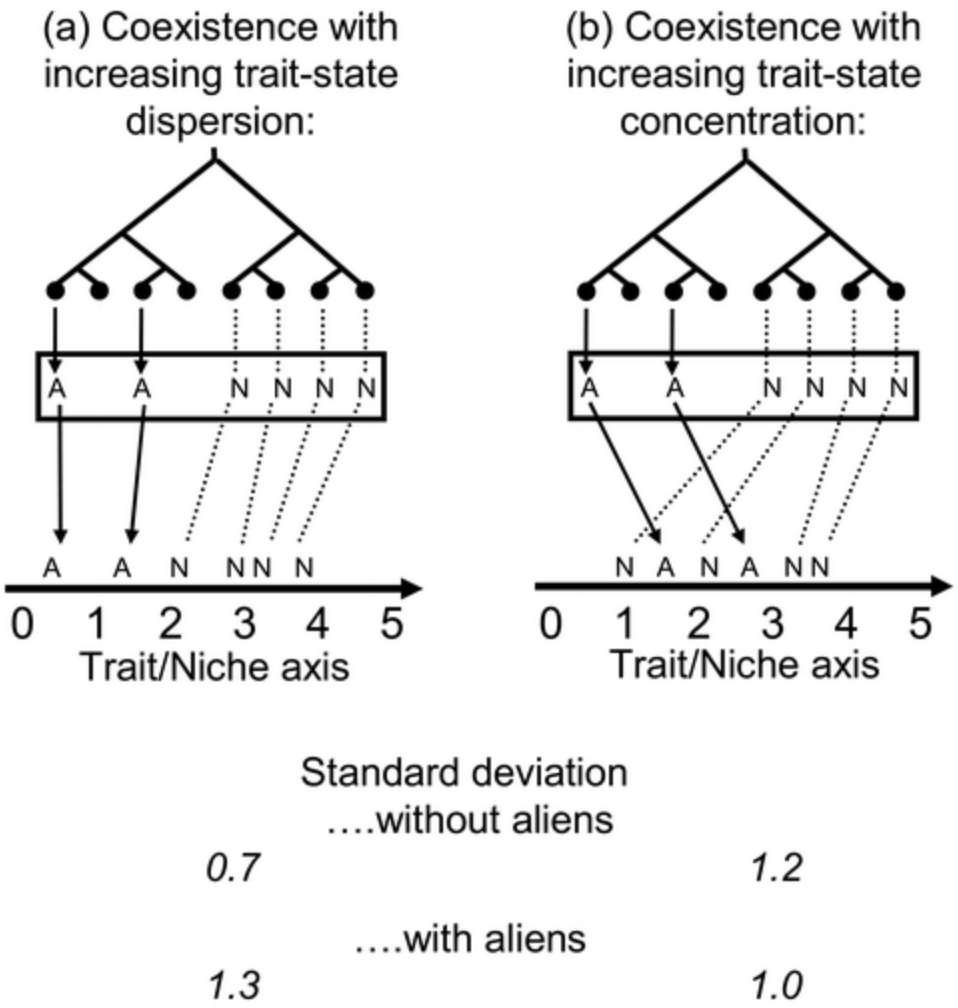

Figure 2. Hypotheses on the relationship between phylogenetic dispersion of local communities and coexistence between natives (*N*) and aliens (*A*). Thin vertical arrows indicate the establishment of alien species in a community. The position of a species along the bold horizontal arrow indicates niche position/trait state. Hypothesis *a*, coexistence of native and alien species with increasing trait-state dispersion; phylogenetically underdispersed communities are underdispersed in niche positions/traits states ("Introduction"). Alien species belonging to distant lineages bring in new niche positions/trait states, thus increasing the standard deviation of niches/trait states. Hypothesis *b*, coexistence of native and alien species with increasing trait-state concentration; phylogenetically underdispersed communities are overdispersed in niche positions/traits states ("Introduction"). Alien species belonging to distant lineages bring in niche positions/trait states similar to those already established, thus decreasing the standard deviation of niches/trait states.

Here we combine recent, large, and unique databases on plant communities, proportions of alien species, functional traits, and phylogenetic positions of plant species. From these data sets we test our central hypothesis that phylogenetically poor communities harbor a higher proportion of alien species. We test this prediction by correlating the average local proportion of aliens across all sites per community type with the corresponding average local phylogenetic dispersion of sites without aliens. In our study of averages within community types, our spatial scale is the local vegetation sample plot, which is the scale relevant to plant interactions and where the presence of alien species might most negatively influence native species (Stohlgren et al. 1999). We also test the respective predictions of the replacement hypothesis versus the coexistence hypothesis and the predictions of the coexistence with increasing trait-state dispersion hypothesis versus the coexistence with increasing trait-state concentration hypothesis, as outlined above. We account for the covarying factors species richness and environmental conditions.

Materials and methods

Characterizing Community Types

We used the Dutch Vegetation Database to describe community composition (Schaminée et al. 1995–1999). We analyzed data of species presence/absence in 7,152 sample plots (for details on selection of plots, see Prinzing et al. 2008) divided into 201 community types, with 1,329 plant species including 116 aliens. Community types were defined as phytosociological associations (Schaminée et al. 1995–1999; for a list of community types and the ranges of proportions of aliens observed, see app. A). We note that some community types are highly anthropogenic, that is, young, which seemingly excludes evolutionary mechanisms suggested by the replacement hypothesis. But even these community types reflect environmental conditions that may have existed for many millions of years, such as trampling. Also, we do not imply that community types are closed units, but categorization is needed as a tool to sufficiently portray the existing diversity and complexity of different environments and their incumbent communities.

Aliens were defined as those that arrived in the Netherlands after 1500 AD (i.e., neophytes; Statistics Netherlands, http://www.milieuennatuurcompendium.nl/tabellen/nl139802a.html) and that have established beyond their specific site of introduction (Ozinga et al.2005); that is, they have established at various localities within the Netherlands. This corresponds to the definition of “invasives” by Richardson et al. (2000) but is contrary to that of *Federal Register* (1999). The latter stresses the replacement of natives by invaders, which in this study is one of the hypotheses to be tested and can thus not be assumed from the outset. Most of these aliens originate from outside central or western Europe (see above Web site). Trees (only 33 species) were excluded, as they are planted mostly in the Netherlands.

For each community type, we quantified the phylogenetic dispersion in plots without aliens (1,284 plots without alien species in total and at least 11 per community type) to reflect the phylogenetic dispersion of the community type before the establishment of aliens. We defined the receptiveness of a community type to aliens as the average proportion of aliens across all plots of that community type and alien establishment as the average proportion of aliens in plots with aliens (i.e., no 0 values). Note that lower richness may increase the variance of proportion estimates (presence or absence of a single alien may strongly increase or decrease the proportion score) but will not affect the mean tendency.

We note that for a given community type, the phylogenetic dispersion of plots without aliens does not necessarily equal phylogenetic dispersion of plots with aliens before the arrival of the aliens. Abiotic conditions may vary to a minor degree even within community types. This variation might cause variation in both the receptiveness to aliens and phylogenetic dispersion. In that case, past alien-free phylogenetic dispersion of plots that received many aliens would be different from present phylogenetic dispersion of plots that received no aliens. We cannot exclude the possibility that this difference varies systematically between community types of high mean phylogenetic dispersion and those of low mean phylogenetic dispersion. The tested correlations between mean present phylogenetic dispersions of plots that received no aliens and mean receptivenesses to aliens thus need to be interpreted with

some caution; we stress that our study tests whether correlative patterns are consistent with our hypotheses but it is not a strict test of the hypotheses themselves.

We measured phylogenetic dispersion as the dispersion of the species represented in a local community across lineages, that is, phylogenetic nodes, represented in a species-level phylogeny of the regional species pool (see Prinzing et al. 2008, which also compares this approach to alternative methodologies). The phylogeny of the species pool was based on the phylogenetic topology for higher plants of central Europe taken from Klotz et al. (2002; checked against Bremer et al. 2003 and Davies et al. 2004). This topology covers 97% of the species in the above phytosociological database. The degree of dichotomous resolution is high (70%), which is essential to resolve phylogenetic patterns of coexistence within a given regional species pool and even within a given habitat type (Cavender-Bares et al. 2006). In fact, in >99.5% of the local communities, >95% of the nodes between the root and the species represented in a community were dichotomies. We calculated phylogenetic dispersion as the standard deviation (SD; i.e., we measured dispersion by using the same units as the data) of the number of species per phylogenetic node multiplied by −1 (Prinzing et al. 2008). In communities of closely related species, few phylogenetic nodes subtend many species, while multiple other nodes subtend no species. This gives a high SD of species numbers per nodes and, multiplied by −1, a low score of phylogenetic dispersion. Alternatively, if species are equally dispersed across the phylogeny, most nodes subtend an intermediate number of species, resulting in a low SD and high phylogenetic dispersion. Phylogenetic dispersion might change as a function of species richness; thus, we standardized the observed dispersions for a null expectation for a given level of species richness (as in Prinzing et al. 2008: (observation − mean null expectation)/(SD of null expectation)). We consider our parameter particularly useful when analyzing topologies (Prinzing et al. 2008) but acknowledge that analyses based on average pairwise phylogenetic distances between species within communities (Warwick and Clarke's [1998] taxonomic distinctness applied to a phylogenetic topology; Webb 2000) led to the same conclusions. Both parameters characterize the dispersion of species across a phylogeny (Hardy and Senterre 2007).

We estimated the abiotic conditions in the plots without aliens on the basis of habitat requirements of the constituent species for light, temperature, soil moisture, soil pH, soil nutrients (from Ellenberg et al. 1991; these Ellenberg values have been extensively confirmed by direct measurements [for a review, see, e.g., Hill and Carey 1997; Diekmann 2003]), and soil salinity (from Schaminée et al. 2007). For each community type, we quantified both the mean and the variation, that is, the SD. We standardized the SDs for a null expectation of random communities of the same species richness (as in Prinzing et al. 2008; (observed − mean − expected)/(SD − expected)). Finally, we characterized the disturbance regime on the basis of mean disturbance strategies of species (from Klotz et al. 2002). We note that such indirect estimations of environment based on species' requirements should be used as relative rather than absolute estimates and should be applied with caution if the main focus is a species-environment relationship (which was not the case in this study).

We analyzed 16 traits considered related to the type of resources used, either directly or indirectly, or to other axes of niche differentiation (table 4), during either the established phase or the dispersal phase. Trait data were taken from the databases BIOPOP (Poschlod et al. 2003) and LEDA (Kleyer et al. 2008). Detailed explanations of the trait data and databases are given in appendix B; we note that all of these traits are phylogenetically conserved (Prinzing et al. 2008). We did not pursue multivariate measures of trait-state dispersion across species because different traits may show distinctly different patterns (Prinzing et al. 2008). Because seed weight varied for six orders of magnitude, we ln transformed it. Plant height varied much less (10%–90% percentiles within one order of magnitude), and other traits were ordinal or categorical. Dispersion of the trait states of a given trait was analyzed as the SD for continuous or ordinal traits and as Simpson's diversity index in its natural logarithm (Rosenzweig 1995) for categorical traits. We standardized observed values for a null expectation on the basis of random communities of identical species richness: (observed − mean − expected)/(SD − expected) (Prinzing et al. 2008).

Statistical Analyses

To test predictions of our core hypothesis, we related phylogenetic dispersion of community types to their receptiveness to aliens by using linear regression analysis (supplemented by nonparametric analysis in the case of nonnormal or heterogeneous distribution of residuals). We note that the results of this analysis did not depend on the invasive or noninvasive status of the aliens. Only 5% of the 116 alien species are classified as invasive aliens according to the DAISIE Web site (http://www.europe-aliens.org/), and these six species characterize (i.e., are found in ≥20% of the plots) only 4% of the 201 community types. Excluding this 4% of communities did not influence the results ($r = -0.382$ vs. initially $r = -0.380$) nor did restriction to only these communities ($r = -0.67$).

We then tested, using multiple regression with stepwise backward exclusion (threshold $P = .05$), whether the relationship between phylogenetic dispersion and receptiveness to aliens persists after including the above-mentioned covariates (species richness, means, and variation of environmental conditions). As phylogenetic dispersion may be related to the presence or absence of particular dominant lineages, we also included the proportion of species belonging to Poaceae and the proportion of species belonging to Fabaceae as covariates. Poaceae is the most species-rich family and is thus numerically dominant. Fabaceae are considered by some authors to dominate plant community assembly by fixing nitrogen (Maron and Connors 1996). The model did not take into account the degree to which communities are influenced by human impacts, in particular by anthropogenically induced seed rain of aliens. Therefore, we completed a separate analysis with an additional covariate that ranked the anthropogenic impact versus the naturalness of the community types; this covariate was available for 125 of the 201 community types (Schaminée and Hennekens 2003).

The replacement hypothesis and the coexistence hypothesis both predict aliens to increase phylogenetic dispersion in underdispersed communities in comparison to communities that are already phylogenetically overdispersed in the absence of aliens. To test this prediction, we quantified for each community type the inferred change in phylogenetic dispersion corresponding to the establishment of aliens as the difference between average phylogenetic dispersion in plots containing aliens and in plots without

aliens. We then tested whether in phylogenetically poor community types, as compared to phylogenetically rich community types, plots of high alien establishment showed increased phylogenetic dispersion. We accounted for the fact that the alien establishment (i.e., considering only plots with aliens) can vary drastically. We hence used multiple regression analysis with difference in phylogenetic dispersion as the dependent variable and alien establishment, phylogenetic dispersion, and the alien establishment × phylogenetic dispersion interaction term as independent variables. To account for possible statistical effects of species richness before the arrival of aliens, we also included richness in plots without aliens in the model (which did not affect the conclusions).

The replacement hypothesis and the coexistence hypothesis make opposite predictions on the effect of aliens on native species richness. To test consistency with these predictions, we quantified for each community type the proportional difference of native species richness between plots with and without aliens: (average native species richness in plots containing aliens − average native richness in plots without aliens)/(average native richness in plots without aliens) (alternative definitions lead to the same conclusions). We then analyzed how phylogenetic dispersion in plots without aliens modifies the statistical effect of alien establishment on the proportional difference of native species richness due to aliens. In other words, we tested whether invaded plots have larger or smaller native species richness than uninvaded plots. We used multiple regression analysis with proportional difference in native species richness as the dependent variable and alien establishment, phylogenetic dispersion, and the alien establishment × phylogenetic dispersion interaction term as independent variables. To account for possible covariation between native richness and alien establishment, we also included native richness without aliens in the model (which did not affect the conclusions).

To test the predictions of the coexistence with increasing trait-state dispersion hypothesis and the coexistence with increasing trait-state concentration hypothesis, we first tested whether in the absence of aliens phylogenetically poor community types have a lower or higher dispersion of trait states. We conducted correlations between phylogenetic and trait dispersion across all 16 traits considered. As these were multiple

tests across multiple, partly intercorrelated variables, we additionally (*a*) applied a sequential Bonferroni correction (Holm 1979) of the *P* values and (*b*) conducted a principal component analysis (based on a correlation matrix; StatSoft 2009) across the 16 variables, and we retained the first axis and correlated this with phylogenetic dispersion. This principal component axis explained 23% of the total variance and was positively correlated with dispersions of 14 of 16 traits; the two remaining negative correlations were very weak (ranked as eleventh and sixteenth, respectively, in strength of the relationship). It could hence be used as a single derived variable in place of the multiple intercorrelated original variables.

Second, we tested whether in phylogenetically poor community types compared to phylogenetically rich community types, alien establishment within plots correlates with a decreased or increased dispersion of trait states. We used multiple regression analysis with difference in trait-state dispersion (plots with aliens − plots without aliens of a given community type) as the dependent variable. As independent variables we included the phylogenetic dispersion, trait-state dispersion, and species richness in plots without aliens, the alien establishment, and the alien establishment × phylogenetic dispersion interaction term. Again we tested trait-state dispersions of each of the 16 traits individually (with and without sequential Bonferroni correction) and then tested the first axis of the above principal component analysis across trait-state dispersions of all 16 traits.

Results

We found that average phylogenetic dispersion (in the absence of aliens) and receptiveness to aliens (average proportion of alien species across all plots) varied considerably among community types (fig. 3). Apparently ecologically similar community types could be ranked from very low to very high in phylogenetic dispersion (e.g., the wetland communities *Ericetum tetralicis* and *Ranunculo–Senecionetum juncetosum articulati* yield phylogenetic dispersions of −3.4 and 7.2, respectively). This indicates that phylogenetic dispersion was not an abstract measure of an obvious pattern in variation across communities.

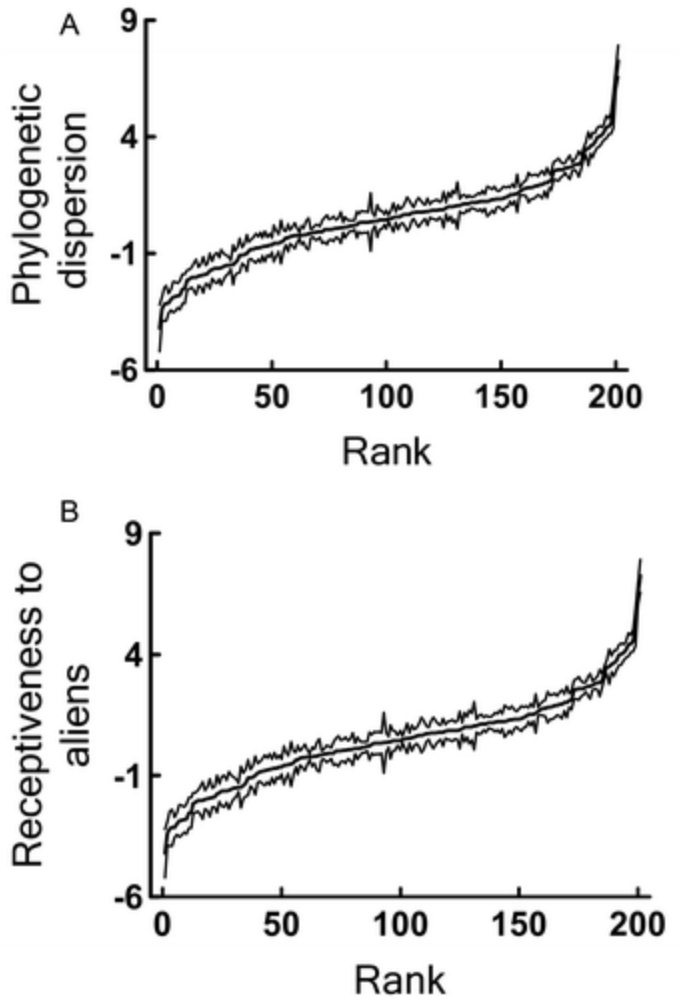

Figure 3. *A*, Average (±SE) phylogenetic dispersion of 201 community types in plots without aliens. *B*, Average (±SE) receptiveness to aliens of different community types (i.e., proportion of aliens across all plots). Community types are ranked from smallest to largest.

Low phylogenetic dispersion correlates with increased receptiveness to aliens. We found that community types with a low phylogenetic dispersion (in the absence of aliens) harbor a higher proportion of alien species ($t_{1,199} = -5.8$, $P < .0001$; fig. 4*A*). A multiple regression accounting for the influence of species richness, the means and variation of abiotic conditions (light, temperature, soil moisture, soil pH, soil nutrients, and soil salinity), the disturbance level, and the presence of Poaceae or Fabaceae confirmed these results ($t_{1,192} = -3.14$, $P = .002$; fig. 4*B*; table 1). The stepwise backward regression excluded species richness (which in itself is a good predictor of

receptiveness to aliens; $r = -0.41$) and retained variables strongly correlated with richness such as pH or the variability of light, moisture, soil nutrients ($r > -0.40$ to −0.75), or temperature ($r = 0.24$). These may thus be the environmental factors that ultimately contribute to the univariate relationship between richness and receptiveness to aliens. Note also that inclusion of anthropogenic impact into the model did not change the effect of phylogenetic dispersion ($t_{1,116} = -2.43$, $P = .016$).

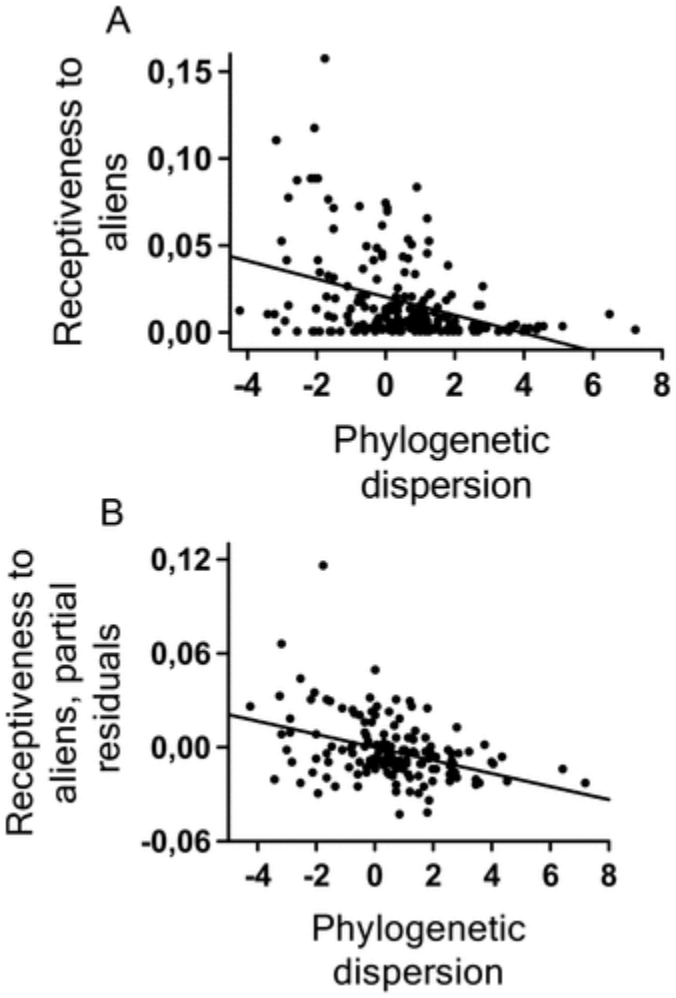

Figure 4. Relationship between average phylogenetic dispersion of community types and average receptiveness to aliens (terms defined in fig. 3: *n* = 201). *A*, Simple regression ($r_p =$ -0.38, *P* = <0.0001; note that nonparametric analysis leads to the same result: $r_s =$ -0.34, *P* = <0.0001). *B*, Partial residuals from multiple regression accounting for the influence of species richness, means and variation of abiotic conditions (light, temperature, soil moisture, soil pH, soil nutrients, and soil salinity), disturbance level, and the presence of Poaceae and Fabaceae (partial correlation: *r* = -0.23, *t* = -3.14, *P* = 0.002).

Table 1: Stepwise backward regression analysis explaining variation in receptiveness to aliens of community types by phylogenetic dispersion and multiple ecological characteristics of plots without aliens

	β	$t_{1, 192}$	P
Phylogenetic dispersion	.23	−3.14	.0019
Mean light	−.62	−6.34	<.0001
Variation in light	−.34	−3.52	.0005
Mean temperature	.18	2.14	.0335
Variation in soil moisture	.22	2.82	.0053
Mean soil pH	−.40	−4.72	<.0001
Variation in soil pH	−.21	−2.53	.0122
Variation in soil nutrients	.31	4.02	<.0001

Note: Variables excluded from the model were variation in mean temperature, mean soil moisture, mean soil nutrients, mean soil salinity, variation in soil salinity, species richness, proportion of Poaceae, and proportion of Fabaceae. (Note that species richness was included as a covariable and deleted by the model.) N = 201 community types, r^2 = 0.46, F = 20.74, P < .0001. See also figure 4*B*.

Low phylogenetic dispersion in plots without aliens correlates with an increased phylogenetic dispersion in plots with high alien establishment.

Overall we found that higher alien establishment (i.e., average proportion of aliens across plots with aliens) correlated with a decrease in community phylogenetic dispersion. This trend, however, was greatly reduced in community types that were phylogenetically underdispersed in the absence of aliens (negative interaction term in table 2). This pattern is consistent with the following scenario: everything else being equal, phylogenetically underdispersed communities gain more lineages or lose fewer lineages as a result of the establishment of aliens than do phylogenetically overdispersed communities. This confirms the assumption of both the coexistence with increasing trait-state dispersion hypothesis (fig. 2*a*) and the coexistence with increasing trait-state concentration hypothesis (fig. 2*b*).

Table 2. Regression analysis explaining differences in the mean phylogenetic dispersion of community types between plots with aliens and plots without aliens.

	β	$t_{1, 166}$	*P*
Phylogenetic dispersion	.44	2.38	.0182
Species richness	−.58	−4.12	<.0001
Alien establishment	−.89	−8.80	<.0001
Phylogenetic dispersion × alien establishment	−.81	−6.15	<.0001

Note. Independent variables are alien establishment (average proportion of aliens across plots with aliens), average phylogenetic dispersion and average species richness in plots without aliens, and the interaction between phylogenetic dispersion and alien establishment. Phylogenetically underdispersed community types tend to gain proportionally more (or lose less) native species due to alien establishment than do phylogenetically overdispersed communities. N = 171 community types with aliens, r^2 = 0.35, F = 22.7, P < .0001.

Low phylogenetic dispersion in plots without aliens correlates with coexistence between aliens and natives in plots with high alien establishment.

We found that the statistical effect of alien establishment on the gain or loss of native species across community types strongly depended on the community types' phylogenetic dispersion in the absence of aliens. The general relationship between alien establishment and the corresponding proportional change of native species richness was negative (table 3), but with declining phylogenetic dispersion, this negative relationship became increasingly positive (negative interaction between phylogenetic dispersion × alien establishment; table 2). This relationship is clarified in figure 5: in phylogenetically rich community types, the establishment of only a few aliens correlates with a reduced native species richness, whereas in phylogenetically poor communities, such reduced native species richness is observed only where large proportions of aliens establish. In general, in the phylogenetically poor community types, alien establishment even corresponds to the establishment of additional native species (note that differences in initial native species richness of the community types are taken into account in the analysis in table 2). This result confirms the prediction of the coexistence hypothesis (fig. 1*B*) and contradicts the replacement hypothesis (fig. 1*A*).

Table 3. Regression analysis explaining variation in the proportional difference of species richness between plots without aliens and plots with aliens across community types

	β	$t_{1, 166}$	*P*
Phylogenetic dispersion	.35	2.10	.038
Species richness	−.58	−5.94	<.0001
Alien establishment	−1.09	−14.65	<.0001
Phylogenetic dispersion × alien establishment	−.78	−9.58	<.0001

Note. Independent variables are alien establishment (average proportion of aliens across plots with aliens), average phylogenetic dispersion and species richness in plots without aliens, and the interaction between phylogenetic dispersion and alien establishment. Phylogenetically underdispersed community types tend to gain proportionally more (or lose less) native species due to alien establishment than do phylogenetically overdispersed communities. $N = 171$ community types with aliens, $r^2 = 0.47$, $F = 36.5$, $P < .0001$. See also figure 5 for an illustration of the interaction term.

Low phylogenetic dispersion in plots without aliens correlates mostly with high trait-state dispersion.

Testing 16 different traits (including niche positions), we found that most traits tended to be overdispersed in phylogenetically underdispersed community types; that is, trait-state dispersion was correlated negatively with phylogenetic dispersion (significantly so for 12 traits, compared to three significantly positive correlations; $N = 201$ community types; app. C). Almost all of these relationships persisted after sequential Bonferroni correction. Also, the first principal component, reflecting overdispersion of most trait variables (see “Material and Methods”), was strongly correlated with phylogenetic clustering ($r = -0.66$, $P < .0001$). This confirms for most traits the assumption of the coexistence with increasing trait-state concentration hypothesis (fig. 2*b*) and not that of the coexistence with increasing trait-state dispersion hypothesis (fig. 2*a*).

Low phylogenetic dispersion in plots without aliens tends to correlate with a decrease in trait-state dispersion in plots with high alien establishment.

We found significant effects of phylogenetic dispersion on the change of trait-state dispersion with increasing alien establishment (i.e., significant interaction terms; table 4) in six traits and a marginally significant effect ($P = .065$) in one further trait. In five of

these traits (light and soil fertility niche, life form, life strategy, and height), phylogenetically underdispersed communities were increasingly underdispersed in trait states where aliens established relative to phylogenetically overdispersed communities; that is, the interaction term in table 4 was positive. In two traits (vegetative reproductive structures and growth form), the trend was the opposite. Sequential Bonferroni correction confirmed one of the positive and none of the negative interaction terms. The first principal component, reflecting overdispersion of most trait variables ("Material and Methods"), showed a distinctly positive interaction term ($\beta = 0.39$, $P = .0091$). Positive interaction terms confirm predictions from the coexistence with increasing trait-state concentration hypothesis (fig. 2*b*); negative interaction terms confirm predictions from the coexistence with increasing trait-state dispersion hypothesis (fig. 2*a*). The strongest relationship found is illustrated in figure 6.

Traits that are particularly overdispersed in phylogenetically underdispersed community types (highly negative correlations between trait-state and phylogenetic dispersion in plots without aliens; app. C) become increasingly underdispersed with the establishment of aliens in such communities (interaction terms listed in table 4): $N = 16$ traits, $r = -0.54$, $P = .03$ (correlations and interaction terms transformed into effect sizes [Fisher's Z_r; Rosenthal 1984] before correlation with each other).

Table 4. Regression analyses explaining differences in trait-state dispersion of community types between plots without aliens and plots with aliens.

	Trait-state dispersion (β, *P*)	**Species richness (β,*P*)**	**Phylogenetic dispersion (β, *P*)**	**Alien establishment (β, *P*)**	**Phylogenetic dispersion × alien establishment (β, *P*)**
Persistence traits:					
Light niche	−.18, .002	.09, .571	−.66, .002	.17, .175	.58, <.001
Soil nitrogen niche	.09, .255	.61, <.001	−.44, .051	.35, .004	.36, .022
Soil moisture niche	−.42, <.001	−.28, .129	.33, .125	.63, <.001	.23, .147
Life span	−.02, .782	.72, <.001	−.59, .011	.05, .654	.18, .242
Height	−.20, .009	−.15, .360	−.25, .227	.08, .491	.28, .065
Life form	−.22, .027	.40, .018	−.54, .016	.43, <.001	.43, .008
Life strategy	.03, .737	.34, .061	−.30, .175	.33, .009	.33, .042
Growth form	.06, .44	−.39, .027	.56, .014	−.17, .182	−.39, .018
Dispersal traits:					
Extent of sexual reproduction	−.41, <.001	.12, .504	−.30, .203	.16, .167	.21, .160
Clonal extension	.16, .051	.06, .751	.11, .623	−.02, .877	−.20, .223
Vegetative reproduction structures	.12, .09	−.61, <.001	.85, .001	−.09, .433	−.433, .006
Seed weight	.69, <.001	.27, .006	−.12, .345	.43, <.001	.08, .403
Diaspore size	−.03, .678	.05, .777	.08, .724	.48, <.001	−.04, .775
Diaspore form	−.08, .357	.12, .480	−.10, .645	.51, <.001	.02, .870
Abiotic dispersal vector	.06, .619	−.14, .469	.21, .351	.21, .095	.08, .642
Biotic dispersal vector	−.05, .635	−.06, .760	−.09, .687	.06, .636	.18, .259
Principal component 1	−.14, .163	.24, .131	−.25, .227	.71, <.001	.39, .009

Note. Independent variables are alien establishment (average proportion of aliens across plots with aliens), phylogenetic dispersion, species richness and trait-state dispersion in plots without aliens, and the interaction between phylogenetic dispersion and alien establishment. Our hypotheses refer to the interaction terms, and those with *P* values <.1 are underlined (after sequential Bonferroni correction, the interaction term for light niche remains at P <.05, and that for vegetative reproductive structure is at at P <.1). The last line gives the corresponding regression model for the first component of a principal component analysis correlated positively with overdispersion of most traits (“Material and Methods”). *N* = 171 community types with aliens. See also figure 6 for an illustration of the interaction term for the light niche trait.

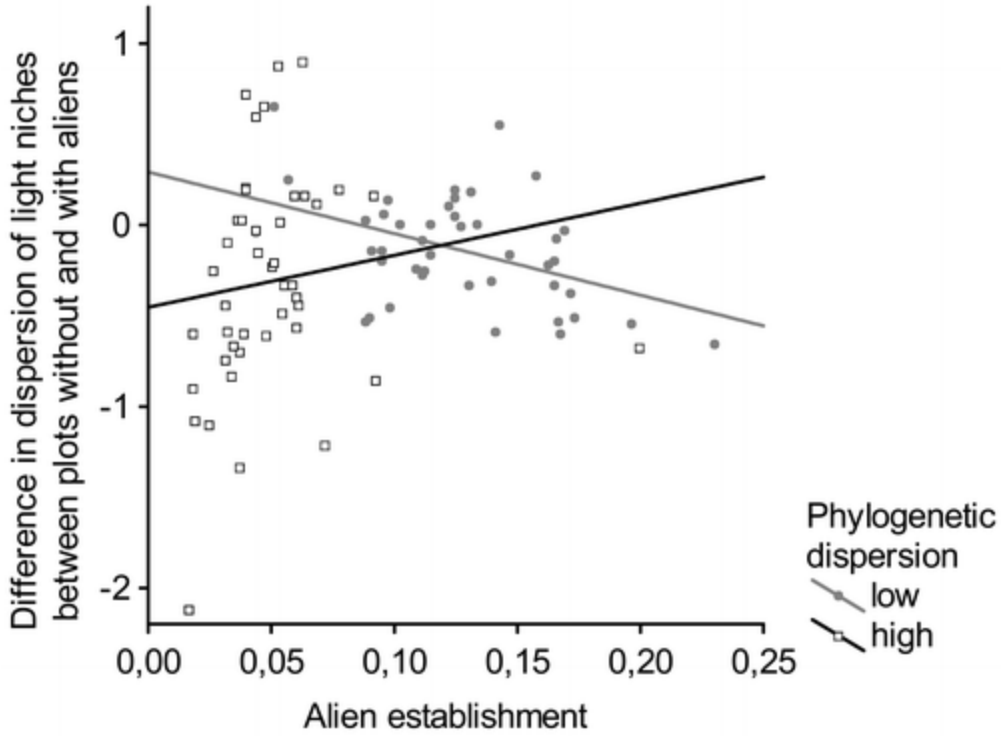

Figure 6. Relationship between alien establishment (see fig. 5) and the change of dispersion of the light niche within communities ("Material and Methods") corresponding to the establishment of aliens. The relationship is positive for community types of high phylogenetic dispersion but negative for community types of low phylogenetic dispersion (lower and higher quartile of phylogenetic dispersions, respectively). For statistical analysis of this and 15 other traits see table 4.

Discussion

Our results show that community types composed of species from phylogenetically distinct lineages (i.e., phylogenetically rich or overdispersed communities) are less receptive to alien establishment. In contrast, community types consisting of closely related species (i.e., phylogenetically poor or underdispersed) are more receptive to aliens. Our results are in accord with patterns previously observed at a biogeographical scale (Darwin 1859). In a manner similar to that of many oceanic islands, particular mainland plant communities across a landscape tend to be phylogenetically poor and more receptive to aliens. However, contrary to the suggestions of Darwin and others for islands, native species in phylogenetically poor mainland communities do not appear to be particularly naive to and easily replaced by alien species. The results indicate that

aliens likely displaced distinctly fewer native species in phylogenetically poor community types than in phylogenetically overdispersed communities. This confirms the hypothesis of coexistence of native and alien species in phylogenetically poor communities (fig. 1*B*). While we cannot exclude future extinctions of natives from some of the plots over a longer timescale, we note that we are studying regionally well-established alien species. The impact of these species on the native flora hence persists already for decades.

Coexistence of native and alien species in phylogenetically poor communities could occur in trait space. The trait space of phylogenetically poor community types was more dispersed for most traits than was that of phylogenetically rich communities. While this confirms the findings of Prinzing et al. (2008), the precise mechanisms explaining this pattern remain unknown and need to be studied. Possible candidate mechanisms are, among others, shared natural enemies or shared metabolic strategies in phylogenetically poor communities (see “Introduction”). In a phylogenetically poor community, alien species that belong to distant lineages would therefore introduce only trait states already present and thus increase the concentration of trait space. Given the differences in physiological strategies and in the associated natural enemies, the aliens are unlikely to have a strong negative impact on the distantly related native species and are therefore unlikely to replace them (for a detailed review of mechanisms, see below and Prinzing et al. 2008). We found correlative evidence that establishment of alien species in phylogenetically poor community types increased phylogenetic dispersion compared to phylogenetically rich community types; that is, aliens belonged to lineages not yet represented in these community types (see also Strauss et al. 2006). We also found correlative evidence that after establishment of aliens, initially phylogenetically underdispersed communities have a more concentrated trait space; that is, additional species brought in trait states already established (fig. 2*b*). The opposite scenario of increasing dispersion of trait-state space due to aliens (fig. 2*a*) was consistent with patterns found in only two traits; these were the traits that were underdispersed in phylogenetically underdispersed communities. These are thus the traits of very high phylogenetic conservatism (Prinzing et al. 2008). Overall, the phylogenetic conservatism of traits may ultimately determine whether aliens in phylogenetically poor

communities establish by filling up or expanding the trait-state space occupied by natives.

Coexistence of native and alien species in phylogenetically poor communities may occur because of a lack of negative indirect interactions. Most alien species belong to alien lineages (see above). Species from an alien lineage are less likely to share, and hence acquire, the pests, pathogens, and herbivores of incumbent native species (Goßner et al. 2009). Conversely, incumbent native species are less likely to acquire the pests, pathogens, and herbivores of alien species. This paucity of shared negative biotic mediators can reduce apparent competitive interactions between aliens and natives (Holt and Lawton 1994).

Finally, coexistence of native and alien species in phylogenetically poor communities may occur within the species pool; phylogenetically poor local communities recruit from a smaller regional native species pool than do phylogenetically rich, overdispersed communities (Gerhold et al. 2008). Such small species pools could impede the establishment and turnover of native species across local communities within a region and thus facilitate the establishment of alien species introduced from foreign species pools.

We stress that further direct tests of our hypotheses would require experimental control, which was not feasible with our macroecological approach covering all environments in a given region. However, finding consistency of such large-scale patterns with particular hypotheses will increase the focus on these hypotheses and justify future direct tests by small-scale experiments, and it will guide these experiments. For instance, our results may guide the choice of appropriate community types on which alien treatments can be applied, and they would advocate performing experiments with established community types in nature rather than with artificially assembled ones, as in the latter, assembly processes are to a large degree replaced by seeding and weeding.

The hypothesis of coexistence of native and alien species in phylogenetically poor communities might open a new avenue for the enduring debate about community saturation—the idea that biotic interactions limit the number of species within a community and more species-rich communities therefore better resist the establishment

of aliens (going back to Elton 1958). Community saturation in terms of species richness has found little empirical support (Kennedy et al. 2002), and recently the whole concept has been suggested to be rather a myth (Stohlgren et al. 2008). Similarly, there is no good evidence for saturation at the individual level, as species abundances in ecological communities rather covary positively in time, not negatively as expected by competition theory (Houlahan et al. 2007). Our findings support the idea that phylogenetic proximity and the trait states of both present and potential new species are more important in determining the success and outcome of establishment of aliens than just numbers of species per se (see also Starzomski et al. 2008). Thus, it is time to move beyond the saturation concept of species numbers and explore the evolutionary history behind species richness numbers (e.g. Bartish et al. 2010).

Our results have clear implications for the protection of biodiversity and for advancing the field of conservation biogeography (Richardson and Whittaker 2010). The results suggest that consequences of aliens on native richness may vary not only between the local scale and the landscape scale (Knight and Reich 2005) but also at the local scale between communities differing in phylogenetic dispersion. Phylogenetically less diverse communities are more receptive to alien species. Even though these aliens do not reduce native species richness in these phylogenetically underdispersed communities, they represent a door through which alien species can enter into a region. Such communities should thus warrant increased protection from alien species. This knowledge could be used in conservation planning, for example, in the selection of protection areas and in regional conservation programs. Predicting receptiveness to aliens from the phylogenetic dispersion of a community can now be effectively applied, as phylogenies are readily available for many groups of species worldwide (Judd et al. 2002; Klotz et al. 2002; Bremer et al. 2003). Our study has shown that the merging of traditional phytosociological databases with modern phylogenies can be a powerful tool to approach these conservation goals.

Overall, our results help to resolve the long-standing debate on the role biodiversity plays in determining how receptive a community is to aliens. If biodiversity is quantified across the entire tree of life and not just by counting the tips of a tree (i.e., species), increased biodiversity indeed correlates with decreasing receptiveness to

aliens. Strong negative interactions between aliens and incumbent natives, leading to the observed replacement of natives by aliens, may explain why phylogenetically rich communities are less receptive to aliens. Inversely, phylogenetically poor communities might be receptive to aliens because aliens can coexist with natives even if they share similar trait states, leading to an increase of species richness and of trait-state concentration with the establishment of aliens. Such coexistence between functionally similar aliens and natives in phylogenetically poor communities might be favored by their phylogenetic dissimilarity.

Acknowledgments

This study was supported by the Estonian Science Foundation (grants 7610, 8613), the European Union through the European Regional Development Fund (Frontiers in Biodiversity Research Centre of Excellence), the Estonian-French funding program PARROT, a Centre National de la Recherche Scientifique–Action Thématique incitative sur Programme funding, the Biodiversity and Climate Research Centre (BIK-F), and a Purkine Fellowship of Academy of Sciences of the Czech Republic. The manuscript profited from very insightful comments from the referees.

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Appendix A. List of community types and their ranges of proportions of alien species.

Community types are associations obtained from Schaminée et al. (1995–1999). Only community types for which at least 10 plots without aliens were available; hence, the minimal proportion of introduced species is 0 throughout.

Table A1.

Community type	Proportion of introduced species mean (receptiveness to aliens)	Maximum
Ranunculetum baudotii	.013	.143
Potametum lucentis	.052	.200
Myriophyllo-Nupharetum	.041	.154
Stratiotetum	.045	.143
Ranunculetum circinati	.061	.167
Myriophyllo verticillati–Hottonietum	.049	.182
Callitricho-Hottonietum	.043	.125
RG *Potamogeton* pect. en Zannich. pal. ssp. ped.–(*Zannichellietalia pedic.*)	.025	.111
RG *Potamogeton pusillus* en *Elodea nuttallii*–(*Parvopotamion*)	.083	.182
Eleocharito palustris–Hippuridetum	.017	.143
Rorippo–Oenanthetum aquaticae	.020	.091
Sagittario-Sparganietum	.074	.231
Typho-Phragmitetum	.020	.200
Typho–Phragmitetum typhetosum angustifoliae	.010	.143
Typho–Phragmitetum typicum	.005	.111
Caricetum ripariae	.019	.111
Caricetum gracilis typicum	.006	.067
Caricetum paniculatae	.008	.100
RG *Glyceria maxima*–(*Phragmitetea*)	.003	.111
Pallavicinio–Sphagnetum typicum	.000	.000
Equiseto variegati–Salicetum repentis	.000	.000
RG *Carex nigra–Agrostis canina*–(*Caricion nigrae*)	.000	.000
RG *Calamagrostis canescens*–(*C. nigrae*)	.000	.000
RG *Myrica gale*–(*C. nigrae*)	.011	.167
Lycopodio-Rhynchosporetum	.000	.000
Ericetum tetralicis	.010	.125
Sphagno palustris–Ericetum anthoxanthetosum	.036	.143
Plantagini–Lolietum cichorietosum	.008	.042
Plantagini–Lolietum puccinellietosum distantis	.012	.111
Bryo–Saginetum typicum	.015	.111
Ranunculo–Alopecuretum rorippetosum	.006	.083
Ranunculo–Alopecuretum typicum	.000	.000
Ranunculo–Alopecuretum equisetetosum palustris	.000	.000
Ranunculo–Alopecuretum inops	.000	.000
RG *Poa trivialis–Lolium perenne*–(*Plantaginetea majoris/Cynosurion cristati*)	.005	.091
RG *Carex arenaria–Poa annua*–(*P. majoris/Koelerio-*	.005	.063

Community type	Proportion of introduced species mean (receptiveness to aliens)	Maximum
Corynephore)		
RG *Agrostis stolonifera*–(*Lolio–Potentillion anserinae*)	.003	.050
RG *Festuca arundinacea–(Lolio–P. anserinae*)	.004	.047
RG *Agrostis canina–Ranunculus repens*–(*Lolio–P. anserinae/Molinietalia*)	.003	.063
Violo–Corynephoretum typicum	.000	.000
Violo–Corynephoretum koelerietosum	.001	.050
Ornithopodo-Corynephoretum	.015	.100
Festuco–Thymetum jasionetosum	.005	.053
Festuco–Thymetum anthoxanthetosum	.005	.067
Festuco–Galietum typicum	.000	.000
Festuco–Galietum trifolietosum	.003	.031
Medicagini–Avenetum luzuletosum	.004	.049
Medicagini–Avenetum arrhenatheretosum	.000	.000
Phleo–Tortuletum typicum	.008	.118
Phleo–Tortuletum cladonietosum	.001	.071
Phleo–Tortuletum brachythecietosum	.013	.143
Sileno–Tortuletum picridetosum	.007	.107
Tortello–Bryoerythrophylletum typicum	.000	.000
Tortello–Bryoerythrophylletum encalyptetosum	.000	.000
Taraxaco–Galietum cladonietosum	.001	.031
Taraxaco–Galietum typicum	.000	.000
Taraxaco–Galietum fragarietosum	.004	.077
Taraxaco–Galietum plantaginetosum	.002	.048
Anthyllido–Silenetum sedetosum	.002	.053
Anthyllido–Silenetum rhytidiadelphetosum	.002	.032
RG *Aira praecox*–(Koelerio-Corynephoretea)	.000	.000
RG *Euphorbia cyparissias*–(*Koelerio-Corynephoretea*)	.009	.095
RG *Agrostis capillaris–Hypochaeris radicata*–(*Trifolio–Festucetalia ovinae)*	.018	.083
RG *Salix repens*–(*Polygalo-Koelerion*)	.004	.056
RG *Rosa pimpinellifolia*–(*Polygalo-Koelerion*)	.003	.042
Gentiano-Koelerietum	.000	.000
Cirsio dissecti–Molinietum typicum	.003	.037
Crepido–Juncetum acutiflori	.001	.016
Lychnido–Hypericetum typicum	.002	.071
Ranunculo–Senecionetum juncetosum articulati	.001	.019
Scirpetum sylvatici	.003	.059
Arrhenatheretum typicum	.001	.043
Arrhenatheretum festucetosum arundinaceae	.003	.056
Arrhenatheretum luzuletosum campestris	.000	.000
Arrhenatheretum medicaginetosum falcatae	.002	.036
Lolio–Cynosuretum typicum	.001	.042
Lolio–Cynosuretum lotetosum uliginosi	.000	.000
Lolio–Cynosuretum hordeetosum	.000	.000
Lolio–Cynosuretum plantaginetosum mediae	.001	.027

Community type	Proportion of introduced species mean (receptiveness to aliens)	Maximum
RG *Holcus lanatus–Lolium perenne*–(*Molinio-Arrhenatheretea*)	.003	.111
RG *Holcus lanatus–Lychnis flos-cuculi*–(*Molinietalia*)	.002	.043
RG *Festuca rubra–Lotus uliginosus*–(*Molinietalia*)	.003	.043
RG *Juncus effusus*–(*Molinietalia/Lolio-Potentillion*)	.009	.125
RG *Carex disticha*–(*Calthion palustris*)	.000	.000
RG *Alopecurus pratensis–Lychnis flos-cuculi*–(*Alopecurion/Molinietalia*)	.002	.042
RG *Alopecurus pratensis–Elymus repens*–(*Arrhenatheretalia*)	.003	.067
RG *Alopecurus pratensis–Hordeum secalinum*–(*Alopecurion/Cynosurion*)	.002	.040
RG *Anthriscus sylvestris*–(*Arrhenatheretalia*)	.005	.100
Rubo–Origanetum festucetosum arundinaceae	.001	.018
Polygonato-Lithospermetum	.018	.143
Hyperico pulchri–Melampyretum pratensis	.011	.083
Hieracio–Holcetum mollis	.014	.083
RG *Pteridium aquilinum*–(*Melampyro–Holcetea mollis*)	.048	.182
Galio hercynici–Festucetum ovinae	.014	.143
Gentiano pneumonanthes–Nardetum	.000	.000
RG *Deschampsia flexuosa*–(*Nardetea/Calluno-Ulicetea*)	.031	.200
Genisto anglicae–Callunetum danthonietosum	.041	.286
Atriplicetum littoralis typicum	.000	.000
Salsolo–Cakiletum typicum	.010	.125
Elymo–Ammophiletum typicum	.012	.167
Elymo–Ammophiletum festucetosum	.006	.111
RG *Ammophila arenaria–Carex arenaria*–(*Ammophiletea/Koelerio-Corynephoretea*)	.021	.143
Puccinellietum maritimae typicum	.026	.125
Puccinellietum distantis polygonetosum	.000	.000
Isolepido–Stellarietum cardaminetosum	.010	.065
Digitario–Illecebretum digitarietosum	.034	.125
Polygono-Bidentetum	.015	.167
Rumicetum maritimi chenopodietosum	.011	.077
Chenopodietum rubri rorippetosum	.038	.118
Eleocharito acicularis–Limoselletum	.013	.091
RG *Catabrosa aquatica*–(*Bidentetea tripartitae*/Phragmitetea)	.016	.091
Veronico–Lamietum hybridi	.030	.105
Veronico–Lamietum alopecuretosum	.012	.091
Echinochloo–Setarietum typicum	.065	.188
Echinochloo–Setarietum inops	.032	.143
RG *Matricaria recutita–Spergula arvensis*–(*Aperion spicae-venti*)	.021	.091
Balloto–Arctietum typicum	.007	.071
Balloto–Arctietum diplotaxietosum	.015	.105
Echio–Verbascetum typicum	.043	.143
Echio–Melilotetum typicum	.033	.120
Bromo inermis–Eryngietum campestris	.026	.154
Tanaceto–Artemisietum agrostietosum	.013	.118

Community type	Proportion of introduced species mean (receptiveness to aliens)	Maximum
Tanaceto–Artemisietum typicum	.016	.091
RG *Elymus repens*–(*Artemisietea vulgaris*)	.022	.143
Valeriano–Filipenduletum calamagrostietosum	.003	.063
Valeriano–Filipenduletum holcetosum	.001	.042
Valeriano–Filipenduletum symphytetosum	.002	.100
Valeriano–Senecionetum fluviatilis	.008	.100
Soncho–Epilobietum typicum	.006	.125
Soncho–Epilobietum althaeetosum	.000	.000
RG *Eupatorium cannabinum*–(Convolvulo-Filipenduletea)	.000	.000
RG *Epilobium hirsutum*–(Convolvulo-Filipenduletea)	.020	.250
RG *Calystegia sepium–Phragmites australis*–(Convolvulo-Filipenduletea)	.017	.222
RG *Phalaris arundinacea*–(Convolvulo-Filipenduletea)	.008	.086
Claytonio–Anthriscetum caucalidis	.002	.071
Torilidetum japonicae	.000	.000
Alliario–Chaerophylletum geetosum	.013	.111
Alliario–Chaerophylletum galeopsietosum	.010	.143
Alliario–Chaerophylletum inops	.006	.083
Urtico-Aegopodietum	.009	.125
Urtico–Aegopodietum alliarietosum	.005	.067
Urtico–Aegopodietum holcetosum	.004	.063
Urtico–Aegopodietum inops	.021	.214
DG *Populus* × *canadensis*–(Galio-Urticetea)	.006	.071
RG *Urtica dioica*–(Galio-Urticetea)	.008	.167
RG *Petasites hybridus*–(Galio-Urticetea)	.005	.091
Senecioni–Epilobietum ceratocapnetosum	.045	.188
Senecioni–Epilobietum inops	.042	.154
Rubetum grati	.042	.500
Salicetum auritae	.006	.083
Salicetum calamagrostietosum canescentis	.005	.091
Salicetum typicum	.005	.133
Pruno–Crataegetum typicum	.002	.057
RG *Ligustrum vulgare*–(*Berberidion vulgaris*)	.000	.000
Artemisio–Salicetum agrostietosum stoloniferae	.011	.083
Irido–Salicetum menthetosum	.010	.130
Irido–Salicetum alopecuretosum pratensis	.002	.059
Cardamino amarae–Salicetum urticetosum	.004	.136
RG *Urtica dioica*–(*Salicion albae*)	.005	.059
Thelypterido–Alnetum typicum	.003	.125
Thelypterido–Alnetum caricetosum ripariae	.005	.071
Carici elongatae–Alnetum typicum	.005	.077
C. elongatae–Alnetum cardaminetosum amarae	.000	.000
C. elongatae–Alnetum ribetosum nigrae	.006	.200
C. elongatae–Alnetum rubetosum idaei	.021	.111
C. elongatae–Alnetum caricetosum curtae	.013	.100

Community type	Proportion of introduced species mean (receptiveness to aliens)	Maximum
RG *Rubus fruticosus*–(*Alnion glutinosae*)	.014	.077
RG *Carex acutiformis*–(*Alnion glutinosae*)	.004	.063
RG *Urtica dioica*–(*Alnion glutinosae*)	.006	.167
Erico–Betuletum callunetosum	.000	.000
Erico–Betuletum inops	.059	.200
Carici curtae–Betuletum peucedanetosum	.012	.111
C. curtae–Betuletum typicum	.013	.200
RG *Molinia caerulea*–(*Betulion pubescentis*)	.034	.167
RG *Rubus fruticosus*–(*Betulion pubescentis*)	.053	.222
Leucobryo–Pinetum deschampsietosum	.088	.333
Leucobryo–Pinetum vaccinietosum	.052	.286
Leucobryo–Pinetum molinietosum	.088	.286
DG *Rubus fruticosus*–(Dicrano-Pinion)	.077	.333
RG *Eurhynchium praelongum–Pseudoscleropodium purum*–(Vaccinio-Piceetea)	.088	.333
Betulo–Quercetum deschampsietosum	.157	.400
Betulo–Quercetum molinietosum	.087	.333
Fago–Quercetum vaccinietosum	.076	.400
Fago–Quercetum pteridietosum	.110	.333
Fago–Quercetum convallarietosum	.069	.286
Fago–Quercetum molinietosum	.072	.250
Fago–Quercetum holcetosum	.071	.250
DG *Quercus rubra*–(*Quercion roboris*)	.117	.400
RG *Rubus fruticosus*–(*Q. roboris*)	.071	.400
Violo odoratae–Ulmetum inops	.022	.222
Violo odoratae–Ulmetum scilletosum	.050	.227
Fraxino–Ulmetum typicum	.020	.125
Carici remotae–Fraxinetum	.004	.071
Pruno-Fraxinetum	.015	.231
Stellario–Carpinetum typicum	.008	.133
Stellario–Carpinetum allietosum	.000	.000
Stellario–Carpinetum dryopteridetosum	.004	.061
Stellario–Carpinetum oxalidetosum	.021	.111
RG *Anthriscus sylvestris*–(*Ulmenion carpinifoliae*)	.012	.100
RG *Urtica dioica*–(*Ulmenion carpinifoliae*)	.001	.057
RG *Urtica dioica*–(Circaeo-Alnenion)	.008	.125

Appendix B. Explanations and References for the 16 Traits Analyzed

Traits related to the established phase (persistence traits) are as follows:

1. Moisture demands under field conditions according to indicator values by Ellenberg et al. (1991). These values rank species distributions in the field in 12-step ranks along a moisture gradient and were available for 1,178 species. Although originally based on expert knowledge accumulated from several thousand original studies, Ellenberg indicator values for moisture have meanwhile been extensively validated (references in Prinzing et al. 2001). The same applies to Ellenberg indicator values for nutrients and light (see below).

2. Nutrient demands under field conditions (nine ranks) according to Ellenberg et al. (1991; available for 1,122 species; see above).

3. Light demands under field conditions (nine ranks) according to Ellenberg et al. (1991; available for 1,122 species; see above).

4. Plant height, related to vertical niche differentiation, calculated as the mean between minimal and maximal heights, given in Van der Meijden (1996).

5. Growth form categories according to Barkman (1988): 20 categories characterizing rooting and stem and foliage patterns relating to vertical and horizontal differentiation of occupation of habitat space.

6. Life form according to Raunkiaer (1934): nine categories, and various combinations thereof, relating to differences in seasonal persistence and successional distribution, that is, to habitat use in time (information available for 1,290 species; Klotz et al. 2002).

7. Life strategy categories according to Grime et al. (1987) related to differentiation in the use of habitats and the speed and competitiveness employed in occupying them (competitive, ruderal, stress tolerant, and various combinations thereof; available for 1,196 species).

8. Life span (Klotz et al. 2002), that is, the speed and permanence of establishment within habitats (annual, biannual, perennial with a single generative reproduction and death thereafter, truly perennial with multiple generative reproductions; ranked 1–4); in the case of multiple assignments, the mean between the minimal and maximal values was taken. Information was available for 1,290 species.

Dispersal traits were as follows:

1. Ln seed weight, that is, the amount of resources made available for the embryo and seedling (available for 1,080 species; Poschlod et al. 2003; Kleyer et al. 2008).

2, 3. Diaspore size and diaspore form, related in various ways to the mode and distance of primary and secondary dispersal. We compiled information on length, height, and width of diaspores of 932 species from the literature; in the case of multiple data for a species, we calculated means across minimal and maximal values (Poschlod et al. 2003; Kleyer et al. 2008). We calculated a principal component analysis across this information and extracted the first and second factors, which accounted for a total of 93% of the variation. The first factor was correlated with an increase in length, height, and width, and we thus used scores along the first factor as a proxy for diaspore size. The second factor corresponded to increasing slenderness of diaspores, and we thus used scores along this factor as a proxy of diaspore form.

4. Extent of sexual reproduction, related to the capacity to occupy habitats by short-distance (vegetative) or long-distance (sexual) dispersal: four ranks from 0, no sexual reproduction, to 4, exclusively sexual reproduction (Klotz et al. 2002).

5. Morphological structures of vegetative reproduction, relating to the mode of vegetative occupation of patches. Categories included runner, bulbil, bulb, fragmentation, rhizome, root shoot, shoot tuber, turio, various modifications and combinations thereof, and none (Klotz et al. 2002).

6. Extent of clonal spread in space and time, related to the pace of patch occupation through dispersion, grouped into four categories: spatial clonal dispersion for≤/>10 cm combined with temporal clonal dispersion for ≤/>1 year (Kleyer et al. 2008).

7. Seven abiotic dispersal modes and combinations thereof related to the capacity to use different vectors to spread out and arrive at different types of microsites (Bonn et al. 2000; Poschlod et al. 2005; Römermann and Tackenberg 2005).

8. Seven biotic dispersal modes and combinations thereof; as above for abiotic dispersal modes (Bonn et al. 2000; Poschlod et al. 2005; Römermann and Tackenberg 2005).

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Apendix C. Correlations between mean phylogenetic dispersion and mean trait-state dispersion across plots without aliens of 201 community types.

Pearson correlation coefficients are given. All correlations are significant at $P < .05$ except where indicated (ns; when applying sequential Bonferroni correction across individual traits, seed weight is nonsignificant also). The last line gives the correlation of phylogenetic dispersion to the first component of a principal component analysis calculated across dispersions of all traits ($P < .001$). This principal component is correlated positively with overdispersion of most traits ("Material and Methods").

Table C1.

Trait	***R***
Light niche	−.38
Soil nitrogen niche	−.34
Soil moisture niche	−.64
Life span	−.49
Height	.17
Life form	−.57
Life strategy	−.57
Growth form	−.1 (ns)
Extent of sexual reproduction	−.41
Clonal extension	.38
Vegetative reproductive structures	.18
Seed weight	−.15
Diaspore size	−.38
Diaspore form	−.56
Abiotic dispersal vector	−.31
Biotic dispersal vector	−.31
Principal component 1	−.66

Chapter 12

A new index of functional redundancy, and an examination of the trends of redundancy in ecological communities.

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Abstract

Functional redundancy is a significant aspect of community structure: a concept formulated to prioritize conservation efforts but now seen as having wider significance. However, measurement of functional redundancy has not been standardised, and comparison of the redundancy of different communities, and of community types in different habitats, is scarce. A new index of functional redundancy, *FRedund*, is proposed, based on an index of functional diversity FD_{var}, and overcoming the problems met in using functional groups or dendrograms. To test hypotheses of how and where functional redundancy would differ, 15 communities were sampled in an area of southern New Zealand, including the greatest range of habitats that could be found in the locality. Six traits related to leaf function were measured for all species encountered. Although FD_{var} is intrinsically independent of species richness, the more species-rich communities showed greater functional diversity. Functional redundancy, *FRedund*, differed between individual communities and between habitats / community types, being highest in grasslands and lowest in coastal / wetland communities. A hypothesis that functional redundancy would decrease with soil fertility was not supported; the trend was for it to increase. Redundancy also tended to be high in communities in mesic habitats that comprised a high complement of exotic species.

Introduction

The concept 'functional redundancy' was introduced by Walker (1992) as the presence in a community of functionally-similar species. Walker saw redundancy as a means of prioritising conservation efforts by identifying species that provide unique ecosystem functions. It has been controversial whether the concept should be applied to real-world conservation (Baskin 1994), though Walker had seen value in redundancy as insurance against loss of function when some species were lost due to environmental change, as did Naeem (1998) and Jain et al. (2014). Doubt was cast on this concept by Cowling *et al.* (1994), suggesting that redundancy did not necessarily imply community resilience. Moir *et al.* (2010) applied the concept of redundancy to ecological restoration.

Subsequently, the concept of redundancy has been given theoretical significance, distinguishing between the 'rivet hypothesis', under which the loss of each species leads to some loss of ecosystem function, *versus* the 'redundancy hypotheses', under which there is no loss of ecosystem function until a critical level is reached (Ehrlich and Ehrlich 1981; Gitay *et al.* 1996). However, as Lawton (1996) pointed out, no loss at first and then an immediate sharp decrease in function below the point of zero redundancy is unlikely, because sharp breaks are rarely seen in ecological processes. But does redundancy even exist? Cowling *et al.* (1994) suggested that classical ecological theory predicts little or no functional redundancy, and Loreau (2004), defining redundancy strictly as exact functional equivalency, showed analytically that because of competitive exclusion there can be no redundancy in a stable community. Reich et al. (2012) suggested that some apparent evidence for redundancy has been due to the failure to consider established communities. However, ecological species must by definition differ in phenotype and so cannot be identical in function. In the real world it does not seem realistic to expect no redundancy or complete redundancy, we suggest that only relative values of redundancy are ecologically meaningful.

Review of methods for determining redundancy

Many of the methods for calculating redundancy are based on functional groups / functional types / guilds (Gitay *et al.* 1996). For example, Laliberté *et al.* (2010) formed functional groups by multivariate analysis of traits, and calculated redundancy as the species richness in each group. Fonseca and Ganade (2001) used rarefaction, removing species at random and examining the number of functional groups still represented. However, categorising species into functional groups by necessity results in information loss, as demonstrated experimentally by Marquard *et al.* (2009). Moreover, the redundancy value depends on the number of functional groups used, which is essentially arbitrary (Flynn *et al.* 2009). Joner *et al.* (2011) used an experimental approach to functional group redundancy, an excellent idea, but with the same drawback of arbitrary groups and an approach too laborious to apply to a whole community. The convex hull volume in trait space of Jain et al. (2014) avoids using functional groups, though it does not take into account all species, nor, notably, their abundance.

Several authors have suggested that functional redundancy depends on the relation between species richness and functional diversity (e.g. Naeem and Wright 2003), that is, a community with high species richness but low functional diversity can be seen as having high redundancy. Both species richness and functional diversity are determined by independent and mutual processes. For example, the immigration of a species will necessarily increase species richness but will not necessarily increase functional diversity. However, if immigration results in competitive exclusion of a functionally equivalent species, then species richness and functional diversity will remain unaffected. This combination of independent and mutual processes should not influence redundancy values so long as an index of functional diversity is used that is independent of species richness. An index of this type could be used to overcome the problem of arbitrary functional groups, but this has not been done. We suggest, based upon the logic of Naeem and Wright, an index:

$$\text{Functional redundancy } (\textit{FRedund}) = \frac{\text{Species richness}}{\text{Functional diversity}}$$

If species richness is low and functional diversity is high, functional redundancy must logically be low and *FRedund* indeed is (Fig. 1, point X, *FRedund* = 1). If species richness is high and functional diversity is low, functional redundancy must logically be high and *FRedund* is (Fig. 1, point Y, *FRedund* = 50). Functional redundancy should stay constant along a linear relation between functional diversity and species richn ess (Saskai *et al.* 2009), and *FRedund* does (Fig. 1, points 1 and 2, *FRedund* = 5). As species richness increases, there must be an upper limit to functional diversity when all the functional niches in the community are occupied. Above this point, additional species will be functionally redundant, the functional diversity / species richness relation will saturate, and *FRedund* values will increase, as Petchey and Gaston (2002) envisaged (approaching Point 4, Fig. 1).

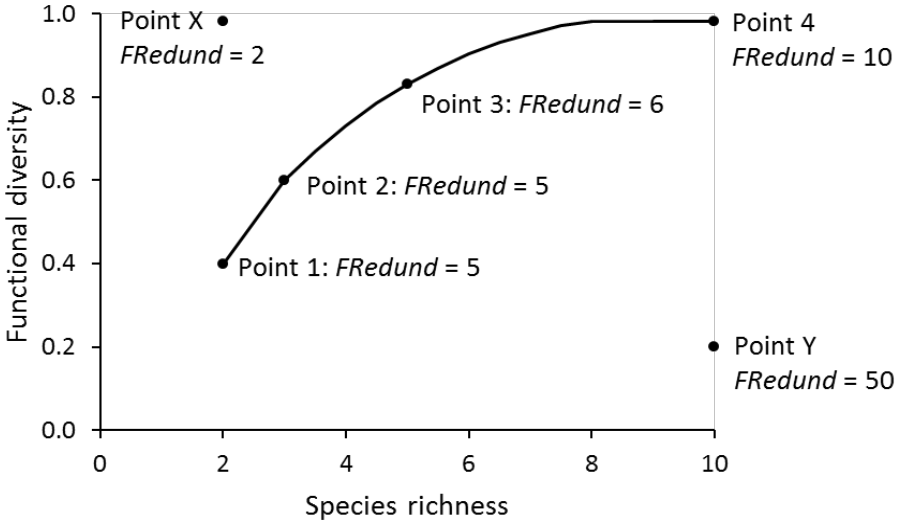

Fig 1. Functional redundancy (index *FRedund*) as determined from functional diversity and species richness.

The choice of index of functional diversity is crucial, for an index that is intrinsically related to species richness, such as dendrogram-based indices (Mouchet *et al.* 2010), will give a spurious relation of functional redundancy with richness. Flynn *et al.* (2009) used comparison with a null model to distinguish genuine relations of functional diversity with species richness from those caused by artefacts of the index. However, it seems preferable to use an index that is independent of species richness in the first place. We use FD_{var} [defined in Appendix 1], an index based on the variance in traits, because it meets this and the nine other requirements for an index of functional diversity enumerated by Mason *et al.* (2003). Thus, no null model is necessary or appropriate. FD_{var} measures a combination of functional richness and divergence (Schluter *et al.* 2010), which was our aim. Mokany *et al.* (2008) found FD_{var} to be the most reliable of the functional diversity indices that they tested. We therefore implement the index of redundancy, *FRedund*, as:

$$FRedund = \frac{\text{Species richness}}{FD_{var}}$$

Where will functional redundancy be higher?

Many authors have concluded that communities contain considerable functional redundancy, though without quantifying it (see Gitay *et al.* 1996; Rosenfeld 2002). Indeed few previous studies have examined differences in redundancy, and where such comparisons exist they have been within a narrow habitat range (e.g. arid shrubland, Cowling *et al.* 1994; rangeland, Saskai *et al.* 2009), and have never employed a rigorous measure of redundancy. The degree to which communities, or habitats with different community types, differ in functional redundancy is therefore unclear. We ask four questions:

1. **Does functional diversity (FD) vary with species richness?** Index FD_{var} is intrinsically independent of species richness, and the null hypothesis would be that as species are added the niche space will remain constant, the added species being within the functional space already occupied, so that FD will not change. de Bello *et al.* (2006) found no significant correlation between FD and species richness across five sites. Otherwise, evidence is sparse, especially as several studies have used FD indices that are intrinsically related to richness, and because species richness is often used as a surrogate for FD in studies of ecosystem function.
2. **Does functional redundancy differ between communities?** This might be expected because of variation in both assembly processes and the evolutionary origin of the species pools. In other studies, differences have been assumed (e.g. Petchey and Gaston 2006; Laliberté *et al.* 2010), in spite of there being very few measurements of redundancy in different communities.
3. **Does functional redundancy differ between habitats** and between the community types that they support? There should be greater differences between habitats and community types in the abiotic and external biotic drivers of community structure, leading to greater differences in redundancy.

4. **Is functional redundancy lower in more fertile habitats?** Abiotic filtering theory predicts that whereas in mesic habitats species with a wide range of strategies can co-exist, in stress habitats many types will be excluded (Grime 2001; Pausus and Verdú 2008). A similar prediction is made from the energy / richness hypothesis, that being in areas with low fertility, and hence low productivity, population sizes will be smaller, so that species occupying marginal functional niches will not persist (Brown *et al.* 2004; Sherratt and Wilkinson 2009). Those marginal functional niches, otherwise low in redundancy, will be empty, leaving higher mean functional redundancy among the remaining species. That is, functional redundancy should be lower in more fertile habitats. Disturbance should have a similar effect in reducing the number of viable niches.

Methods

Communities were sampled at fifteen sites (Table 1) that covered the range of abiotic conditions and habitats present in the Karamea region of the South Island, New Zealand (41º 06′–23′ S, 172º 03′–112′ E). We categorise the communities into three types (Table 1), based on habitat and vegetation physiognomy. The region has a mild climate, with average rainfall of 1802 mm yr^{-1}, and mean air temperatures ranging from 8.6 °C in July to 16.8 °C in February.

In each community, spatially-stratified random sampling was used to locate three 1 × 1 m quadrats, in which shoot frequency of all vascular species was recorded using 100 0.1 × 0.1 m subsquares.

Soil fertility was determined by bioassay (cf. Wheeler *et al.* 1992), a soil sample was taken from 5-10 cm depth in the centre of each quadrat, bulked per community and mixed in a 1:1 ratio with washed sand to eliminate puddling. The mix from each community was separated into three replicate 7 cm × 7 cm × 10 cm deep pots in a heated greenhouse, and 2 g of *Avena sativa* seed sown. After five weeks the shoots were harvested, dried and weighed. The results are expressed as a percentage of the biomass in high-fertility controls that comprised nine pots filled with a 1:1 mixture of potting mix (with ‘Osmocote’ fertiliser NPK 2:1:1) and washed sand.

Table 1. The fifteen communities sampled and the three habitats / community types. Exotic species are indicated by '*'. For further details of the communities see Appendix 2.

Habitat	Community	Major species (physiognomic dominants)	Maximum vegetation height (m)	Species recorded in the site	Percent of species that were exotic
Coastal/ wetland	Lower Saltmarsh	*Samolus repens, Selliera radicans, Juncus maritimus*	1.20	12	25%
	Upper Saltmarsh	*Juncus maritimus, Plagianthus divaricatus. *Schedonorus arundinaceus*	1.58	10	20%
	Coastal Dune	**Ammophila arenaria, Calystegia tuguriorum, *Hypochaeris radicata*	0.80	15	73%
	Jointed-rush Fen	*Apodasmia similis, Coprosma propinqua, Phormium tenax*	2.65	12	8%
	Sedge Fen	*Isolepis distigmatosa, *Agrostis stolonifera, Potamogeton cheesemanii*	0.72	3	33%
	Wooded Fen	*Baumea tenax, Gleichenia dicarpa, Leptospermum scoparium*	2.43	13	8%
Grass-land	Riverbed	*Coprosma robusta, *Lotus pedunculatus, Microlaena stipoides*	2.91	20	30%
	Riverside Driftwood	*Haloragis erecta, *Holcus lanatus, *Schedonorus arundinaceus*	0.73	31	45%
	Rough Grassland	**Holcus lanatus, *Lotus pedunculatus, Paesia scaberula*	1.15	13	54%
	Pasture	**Anthoxanthum odoratum, *Festuca rubra, *Lotus pedunculatus*	0.51	18	67%
Woody	Scrub	*Baumea tenax, Leptospermum scoparium, *Ulex europaeus*	2.50	15	7%
	Beech Forest	*Nothofagus fusca, Podocarpus ferrugineus, Dicksonia fibrosa*	30.41	23	0%
	Beech/ broadleaf Forest	*Nothofagus fusca, Weinmannia racemosa, Metrosideros fulgens*	22.19	17	0%
	Palm Forest	*Rhopalostylis sapida, Metrosideros umbellata, Cyathea dealbata*	35.78	12	0%
	Podocarp Forest	*Dacrycarpus dacrydioides, Melicytus ramiflorus, Ripogonum scandens*	34.56	22	0%

For functional traits, ten shoot samples were collected from each of the species found in any quadrat in each community. To account for between-community ecotypic and plastic differences, those species that occurred in more than one community were separately sampled from each. Six traits were measured, selected to reflect shoot function. Since not all species possessed true laminae, 'leaf' traits were measured on the basis of photosynthetic units (PSUs), the minimum independently-mobile photosynthetic organ most closely functionally analogous to a simple leaf (Smith *et al.* 1994). For species with simple leaves, the PSU was taken to be the leaf lamina, for compound leaves the

leaflet lamina. For ferns, the PSU was defined as the pinna that did not fuse with adjacent pinnae distal of the rachis. For ease of reading, ‘leaf’ is used below. The six traits were:

(a) Support fraction in the terminal shoot: the major function of support tissues is positioning of the leaf area for light capture, but a minor function may be storage (Yang *et al.* 2009). For this purpose, the terminal shoot was defined as a shoot distal of the lowest leaf remaining attached on the main stem. That is, starting at a growing apex, the main stem is followed down until the lowest leaf directly attached to it. For a tree, this was typically in the order of 10-15 cm. The support fraction was defined as the proportion of the total biomass in this terminal shoot that comprised non-photosynthetic material (i.e. non-PSU, i.e, support). Thus, as well as stem, ‘support’ included for forb species the petiole, for compound leaves and ferns the rachis and for graminoids the leaf sheath.

(b) Leaf area: this trait affects light capture, gas exchange, water retention and the dissipation of heat load (Pickup *et al.* 2005). Leaf area was measured with a scanner and Winfolia Pro 2005b software.

(c) Leaf shape: this is implicated in a gas exchange and the dissipation of heat load (Givnish and Vermeij 1976). It was defined as maximum leaf width divided by leaf length.

(d) Lobation: this is another trait affecting gas exchange, water retention and heat load dissipation (Givnish and Vermeij 1976). It was calculated as the ratio of the actual perimeter of a leaf to that of an ellipse of the same length and width, which gives a measure of the extent of lobation.

(e) Specific leaf area (SLA): this trait is widely advocated as the key to plant vegetative strategy, representing tradeoffs between light interception, leaf longevity, defence, etc. (Wright *et al.* 2010). It is defined as the leaf area of an individual leaf divided by its dry weight (drying at 80 °C for 72 hours), making it the reciprocal of specific leaf weight, i.e. of leaf mass per area.

(f) Maximum plant height: this is one of the three characters of the Leaf-Height-Seed (LHS) strategy scheme, and is the crucial component of community stratification (Wright *et al.* 2010).

For further functional justification of these traits see Smith *et al.* (1994) and Stubbs and Wilson (2004).

Functional diversity index FD_{var} (Mason *et al.* 2003; Appendix 1) was calculated for each trait, and the mean taken over the six traits. Log transformations were not needed because they are intrinsic to the calculation of FD_{var}.

Results

Question 1: Does functional diversity vary with species richness?

Functional diversity was higher in communities that were more species-rich (Fig. 2), this represents a true increase in functional diversity with species richness, as index FD_{var} is intrinsically independent of species richness. To examine variation between the quadrats within a community, a joint within-community regression was calculated (i.e. a linear model with FD_{var} as the dependent variate, species richness as independent variate, and the 15 communities as covariates), and it was significant ($R^2 = 0.178$, $P = 0.018$). Thus, the functional diversity / species richness relation holds within communities as well as between. The null model is rejected: functional diversity does increase with species richness, though the scatter in Fig. 2 and the value of R^2 from linear regression show that they are not closely related.

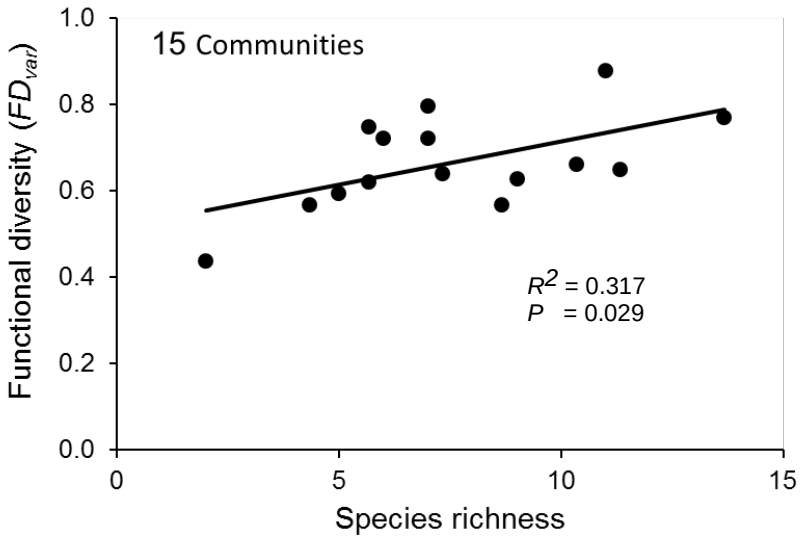

Fig. 2. The relation between functional diversity (the latter as FD_{var} meaned over six traits in 15 communities) and species richness (per 1 × 1 m). The Ordinary Least Squares regression line is indicated.

Question 2: Does functional redundancy differ between communities?

Functional redundancy differed significantly between the 15 communities, tested against the error from the three replicates within each (Table 2; $F_{14,30} = 5.712$, $P = 0.000032$). It was lowest in the Sedge Fen community, and high in two very different communities: Riverside Driftwood and Podocarp (southern hemisphere conifer) Forest. Communities do differ in their functional redundancy.

Redundancy increased with species richness across the communities (Fig. 3), almost proportionally (i.e. almost following a line fitted through the origin, the intercept being only 'marginally significantly' different from 0.0, $P = 0.070$).

Table 2. Mean values of functional redundancy (*FRedund*) in the three habitats and the 15 communities.

Habitat	*FRedund*	Community	*FRedund*
Coastal / wetland	8.72	Lower Saltmarsh	9.65
		Upper Saltmarsh	8.39
		Coastal Dune	8.33
		Jointed-rush Fen	11.48
		Sedge Fen	5.23
		Wooded Fen	9.22
Grassland	15.13	Riverbed	12.52
		Riverside Driftwood	17.29
		Rough Grassland	15.14
		Pasture	15.57
Woody	11.14	Scrub	8.69
		Beech Forest	14.40
		Beech/broadleaf Forest	7.25
		Palm Forest	7.56
		Podocarp Forest	17.78

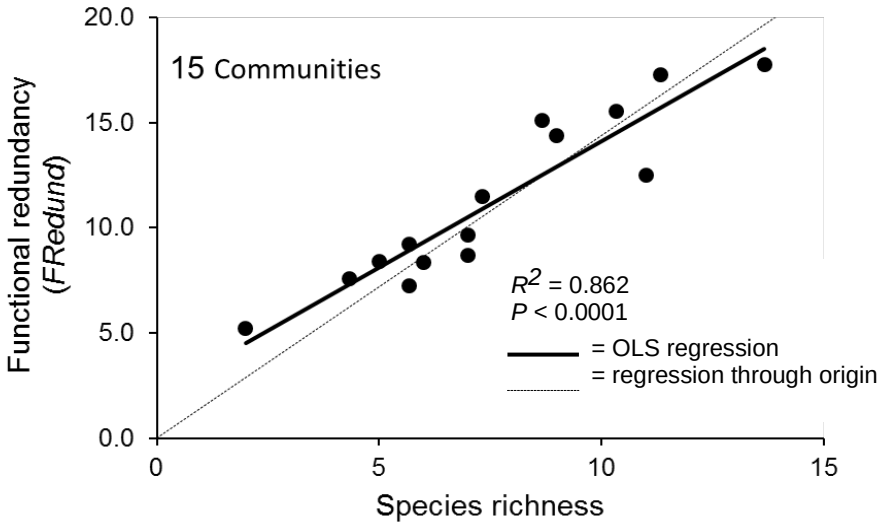

Fig. 3. The relation between species richness (per 1 × 1 m) and functional redundancy (*FRedund*).

Question 3: Does functional redundancy differ between habitats?

The three habitats / community types were significantly different in functional redundancy when tested against the overall error (Table 3). Since there are different communities within each habitat (i.e. communities are nested within habitats), it is more appropriate to test the habitat differences against the community-within-habitat variance, and the habitat differences were significant on this basis too (Table 3). The suggestion that redundancy would differ among habitats is supported. Redundancy was low in the coastal/wetland communities, higher in the Woody communities and highest on average in the Grassland communities. By Tukey's *a posteriori* test, the significant difference was between the Coastal/wetland and Grassland communities.

Table 3. Analysis of variance of differences in functional redundancy (FRedund) between and within the three habitats / community types

Source of variation	SS	DF	MS	Tested against	F	P
Habitat (Coastal/wetland *vs* Grassland *vs* Woody)	296.36	2	148.18	Error	17.92	0.000008
				Community-within-habitat	4.87	0.028
Community-within-habitat	364.86	12	30.40	Error	3.68	0.0019
Error	248.08	30	8.27			
Total	909.30	44	20.67			

Question 4: Is functional redundancy lower in more fertile habitats?

The hypothesis that functional redundancy would decrease with substrate fertility was not supported. In fact the non-significant trend was in the opposite direction (Fig. 4). The greatest outliers were the Pasture and the Riverside Driftwood, both with intermediate fertility but high redundancy. The Podocarp Forest also had high redundancy, though it had the second-highest fertility.

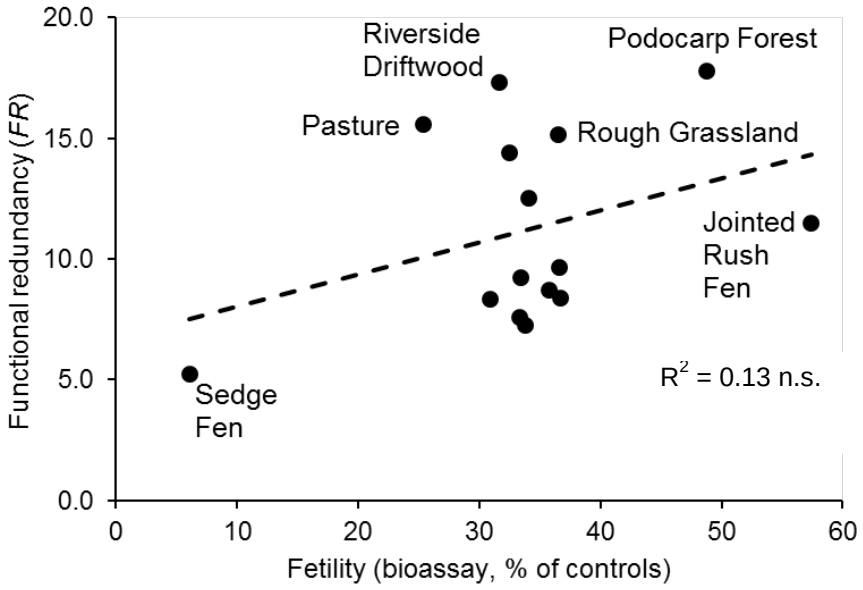

Fig. 4. Relation between functional redundancy (*FRedund*) and fertility (estimated by bioassay, expressed as a percentage of high-fertility controls).

Discussion

Species richness and functional diversity

There has been considerable discussion of a possible correlation of functional diversity with species richness. Petchey and Gaston (2002) and Petchey *et al.* (2007) reported such correlations, but dendrogram-based indices such as their *FD* are intrinsically related to species diversity (Mouchet *et al.* 2010). Petchey and Gaston (2006) argued that this is a realistic aspect of functional diversity. However, others have disagreed, and found it necessary to remove the effect of species richness on *FD* by comparison with a null model (e.g. Flynn *et al.* 2009). Petchey *et al.* (2007) took the same approach of comparison with a null model, but did so graphically.

Avoiding these problems, Cowling *et al.* (1994) used an index free of intrinsic effects of species richness, a variant of the Shannon-Weiner index based on growth form categories. They found in arid and semi-arid southern Africa a positive correlation of functional diversity with species richness, as did Micheli and Halpern (2005) amongst marine algae and animals at 16 sites in California, and Sasaki *et al.* (2009), using a different index, in the plants of Mongolian rangeland. We found a similar relation,

functional diversity rising with species richness. These trends must mean that either: (a) there is a relatively constant number of niches, but when more species are able to tolerate the particular environmental conditions, i.e. to pass the abiotic filter, more of the niches can be filled, or (b) in some habitats there are more niches, and therefore more species can co-exist. The latter explanation would apply to the 'Podocarp Forest' if its high functional diversity (0.77, compared to an overall mean of 0.67) is due to its vertical rainforest stratification. However, on this basis we would expect the number of niches to be rather fixed within a community, yet the overall within-community functional diversity / species richness relation is considerable. An explanation that the number of species is due to an external factor such as the size of the species pool, or random internal variation, and that with increasing species richness there are more functional types owing to a sampling effect, seems to be ruled out by the FD_{var} index we used. Why should additional species extend the range of a trait and increase FD_{var} rather than having a mean value and reduce it?

It seems possible that additional niche differences within communities would have been exposed by using more functional traits. Petchey and Gaston (2006) found that using their functional diversity index, *FD*, the number of traits could systematically alter the level of functional redundancy. This problem does not affect our results because the FD_{var} values were meaned across traits. Thus, although the FD_{var} values and therefore the *FRedund* values could change somewhat if new traits were added, they would not change systematically up or down. The traits we measured are often considered significant, they were measured on field samples, relevant to the site rather than taken from floras or databases, and six traits is a typical number (e.g. cf. Wacker *et al.* 2009). A dataset such as the 23 functional traits of Wilson and Stubbs (2012) would be ideal, but difficult to achieve for the 15 communities used here, so the possibility that our traits do not capture all relevant niches must temper our conclusions.

Is functional redundancy high or low, and where?

There have been some claims of systems having redundancy that is notably high or low overall. Clarke and Warwick (1998) observed soft-bottomed faunal macrobenthos at a location in France and concluded that redundancy was "remarkably high". They, followed

by Guzmán-Alvis and Carrasco (2005), had used heuristic search to form species subsets that were able to reproduce change in a community through time, as judged by rank correlation of similarities to those from the full analysis. This is a different concept of redundancy from that used by other workers. Fonseca and Ganade (2001), with a selection of plant species from a climatic gradient in Argentina, also used a species subtraction approach, but judged the effect on the number of functional groups remaining, and similarly reported a "high redundancy level", though with caveats.

On the other hand, Micheli and Halpern (2005) concluded there were low levels of redundancy amongst multiple trophic levels in rocky marine reefs in an area of California, calculating redundancy as the number of species in a number of functional groups. Such a conclusion depends on the number of groups, but their classification seems reasonable. What their result means, in effect, is low species richness. Gamfeldt *et al.* (2008) also reported "quite low redundancy" in a simulated community from a selection of grassland species, and Sasaki *et al.* (2009) suggested low redundancy in some of the rangelands in Mongolia.

It is difficult to compare studies made in different areas, with different groups of organisms, and with functional redundancy measured in quite different ways. Moreover, we do not believe that absolute levels of redundancy will have meaning; the need is for comparisons of relative levels of redundancy in different habitats and community types. There have been few such studies, all of them comparing one or two communities in one type of habitat. Sasaki *et al.* (2009) compared rangelands with different degrees of grazing disturbance and concluded that there was lower redundancy under harsher environmental conditions, but they did not calculate a value for redundancy and the basis of their conclusion is unclear. Cowling *et al.* (1994) reported higher redundancy in the South African succulent Karoo than in the desert and suggested this was because its more benign climate made it easier for species to persist. Sasaki *et al.* (2009) used traits from floras and Cowling *et al.* (1994) used only growth form. Our complement of six traits related to leaf function is typical of work in functional diversity, e.g. the five traits of Wacker *et al.* (2009).

High redundancy seems to occur in spite of opportunities for niche differentiation. For example, the Podocarp Forest would be expected to have a high number of niches

from its structural complexity and from the opportunity for differences between species in demography and height (Southwood 1996; Condit *et al.* 2006; King *et al.* 2006), giving high functional diversity and hence low redundancy, but in fact redundancy was highest here (Table 2, Fig. 4). Before the arrival of humans much of the area would have been covered by podocarp forest, so this community out of our 15 communities has probably had the longest time since disturbance for species to arrive and redundancy increase. The generally low redundancy of the coastal/wetland communities, which are intrinsically in a dynamic state, supports the suggestion by Dickson and Foster (2008) that disturbance is key to low redundancy. The opposing argument for the effect of disturbance is that, in disturbed habitats, marginal functional niches will become non-viable, making some functional types non-viable and indeed restricting the community to ruderal species (Milder *et al.* 2008; Biswas and Mallik 2010), giving low functional diversity and hence high overall redundancy. Indeed, two of the three communities with the highest measures of functional redundancy (Fig. 4) were probably amongst the most disturbed: the Riverside Driftwood (disturbed by channel movement and the deposition of woody debris) and the Pasture (removal of biomass by grazing, fitting exactly the definition of disturbance by Grime 2001). Substrate heterogeneity has also been suggested to provide niches, so functional diversity in the Riverside Driftwood community matching these niches should be high and therefore redundancy low. It is not, though it is true that this effect of heterogeneity is controversial (Reynolds and Haubensak, 2009; Lundholm, 2009). However, the high redundancy in disturbed sites such as the Riverside Driftwood could also be related to a limited time since disturbance in which competitive exclusion could operate.

Moderately high soil fertility could be an additional factor promoting functional diversity, and thus decreasing redundancy (Dickson and Foster 2008), and this was one of our initial hypotheses. However, in these sites the trend was in the opposite direction (Fig. 4), tending to support the concept of Cowling *et al.* (1994) of higher redundancy in more benign conditions, such as the lowland Podocarp Forest here, where competitive exclusion might be less effective. At the high stress end, waterlogging in the Sedge Fen could be seen as a strong environmental filter, leading to the low redundancy seen there, with only three species present: the leafy grass *Agrostis stolonifera*, the leafless sedge

Isolepis distigmatosa and the water dicot with floating leaves *Potamogeton cheesemanii*. This reasoning could explain the low redundancy in the Coastal/wetland habitat as a whole, significantly lower than in the Grassland communities.

Alexander *et al.* (2011) suggested that in mesic environments environmental filtering is less effective for exotic species, since they are largely generalists. This would lead to high redundancy. Indeed, across the communities that we compared, three of the four highest measures of redundancy were in mesic environments that had a high exotic species complement (Fig. 4; Table 1). The main exceptions to this relation are high redundancy in the completely-native Podocarp Forest, and low redundancy in the Coastal Dune, dominated by exotic species, though the latter habitat could be classed as stressed rather than mesic.

Conclusions

We have implemented a standardised index of functional redundancy (*FRedund*) that overcomes the problems previous authors have met using functional groups or dendrograms. Employing *FRedund* we have demonstrated differences in functional redundancy between 15 communities across three habitats. Until now there has been very little comparison of this kind, and no comparison of redundancy in different regions. King *et al.* (2006) suggested that favourable conditions for growth over most of the year might facilitate co-existence and thus allow high redundancy, a suggestion comparable with that of Cowling *et al.* (1994), and supported by our non-significant fertility trend (Fig. 4). The climate of the region we surveyed is quite aseasonal (see Methods above), which suggests the levels of redundancy found may be higher than in continental regions. We anticipate subsequent datasets that include the measurement of further functional traits. Having demonstrated that functional redundancy is a significant and variable component of plant community structure we expect forthcoming measurements of *FRedund* will be able to guide investigation of hypotheses concerning coexistence and the loss of ecosystem function.

Acknowledgements

We thank Norman W.H. Mason for advice, John B. Steel, Rachael H.E. Lawrence Lodge, Kelly Frogley and Jake M. Alexander for comments on a draft.

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Appendix 1: Functional diversity index FD_{var}.

FD_{var} is based on the variance in traits, weighted by the abundance. It is analogous to evenness index *E_{var}* of Smith and Wilson (1996), but it uses the trait value where *E_{var}* uses the abundance, and it uses the abundance to form a weighted mean and also to weight the deviations. It is basically the variance in the trait values of the species present at a site, with the squared residuals weighted by the abundance of the species involved (the 10 is for scaling):

$$FD_{\text{var}} = 1 - \frac{2}{\pi}\arctan\left[10\sum_{s=1}^{S}\left(\left(\ln(x_s) - \overline{lnx}\right)^2 a_s\right) \Big/ \sum_{s=1}^{S} a_s\right]$$

$$\overline{lnx} = \sum_{s=1}^{S}\ln(x_s)a_s \Big/ \sum_{s}^{S} a_s$$

Where:

a_i = the abundance of species i, out of N species

X_I = the character value of species i

The index meets the 10 criteria of Mason *et al.* (2003) for an ecologically-appropriate index:

Criterion 1: Be constrained to a 0 – 1 range and use that range well.
Criterion 2: Reflect the range of trait values present.
Criterion 3: Be weighted by abundance: the contribution of a species to the index should be proportional to its abundance.
Criterion 4: Decrease when the abundance of a minor species with an extreme trait value decreases.
Criterion 5: Not change appreciably when a species present in minute amounts disappears.
Criterion 6: Be unaffected by the units in which the trait is measured.
Criterion 7: Be symmetrical with regard to small and large trait values.
Criterion 8: Be unaffected by the units in which the abundance is measured.
Criterion 9: Be unaffected by the number of species. The number of taxonomic species itself is not relevant to functional diversity.
Criterion 10: Not change when one species is replaced by two with the same trait value as the original and with the same total abundance as the original. The taxonomic identity of the plants is of no relevance in functional diversity, and if two species are functionally identical, they are functionally one species.

Appendix2: Further details of the fifteen study communities.

Type	Community	Site slope (º)	Site aspect (º)	Latitude	Longitude	Altitude (m)	Distance from sea (km)
Coastal/ wetland	Lower Saltmarsh	0	0	41º 15'S	172º 05'E	1	0.5
	Upper Saltmarsh	0.5	204	41º 17'S	172º 06'E	1	1.0
	Coastal Dune	8	74	41º 12'S	172º 06'E	1	0.0
	Jointed-rush Fen	1	114	41º 17'S	172º 06'E	2	1.1
	Sedge Fen	0	0	41º 17S	172º 05'E	2	0.8
	Wooded Fen	1	243	41º 16'S	172º 07'E	90	2.0
Grass-land	Riverbed	0.5	240	41º 06'S	172º 06E	4	0.6
	Riverside Driftwood	4	68	41º 23'S	172º 03'E	2	0.2
	Rough Grassland	4	221	41º 14'S	172º 12'E	60	10.0
	Pasture	0	0	41º 14'S	172º 06'E	4	0.8
Woody	Scrub	2	36	41º 10'S	172º 07'E	18	1.5
	Beech Forest	5	251	41º 14'S	172º 12'E	40	9.5
	Beech/ broadleaf Forest	9	229	41º 10'S	172º 07'E	15	2.0
	Palm Forest	1	27	41º 06'S	172º 06E	10	0.7
	Podocarp Forest	1	134	41º 22'S	172º 05'E	10	1.3

General Discussion

In this thesis I have targeted community assembly processes with the purpose of identifying the direction and strength of the species interactions they encompass. Species interactions depend on the coexistence mechanisms operating in a community. If a plant community is structured by niches then we could expect to identify assembly rules that underlie this structuring (Diamond 1975, Lawton 1987, Wilson 1999, Levine & HilleRisLambers 2009). Together with my colleagues we have examined assembly processes across biodiversity gradients and we have found that assembly rules are evident. Community assembly is non-random and complementary assembly of functional groups occurs regularly. Across richness gradients composition converges in terms of functional richness, species richness and evolutionary relatedness. The purpose of this general discussion is to draw together my varying lines of inquiry and discuss a digest of our findings in a wider contemporary ecological context.

Biodiversity-ecosystem functioning relationships

The majority of the experimental results presented in this thesis are the output of smaller experiments nested in larger experimental biodiversity platforms. Most of the patterns presented here therefore have an underlying theme – response to biodiversity gradients. In reviewing biodiversity–ecosystem functioning relationships as they were understood from large experimental biodiversity platforms (chapter 1) several conclusions could be drawn from the canon of experimental research (Tilman & Downing 1994, Tilman et al. 1997, Hector et al. 1999, Tilman et al. 2001, Hooper et al. 2005, Roscher et al. 2005, Balvanera et al. 2006, Cardinale et al. 2007, Marquard et al. 2009). Various metrics of biodiversity have significant impacts on biomass production (the most commonly measured metric of biodiversity effects) and a number of associated ecosystem processes (Hector et al. 1999, Crutsinger et al. 2006, Cadotte et al. 2008). The manipulation of species richness and functional group composition revealed increasing biodiversity positively impacts broader ecosystem processes, such as nutrient retention, soil sustainability and carbon cycling (Tilman et al. 1996, Balvanera et al. 2006). The concerns we highlighted were the need for more long-term experiments, in systems beyond grasslands, looking at a greater variety of response variables. A recent analysis of biodiversity effects reported from the Jena Experiment,

found that biodiversity effects do not increase over time (Marquard et al. 2013). The impact of biodiversity remained positive, but the increase in effect sizes were no longer linear with time, as previously expected (Roscher et al. 2005, Marquard et al. 2009). The drivers of the positive effect seem to be a revolving subset of species that perform better in more species rich communities, driving overyielding (Marquard et al. 2013). The mechanism of turnover was suspected to be negative density dependant growth rates of species, a stabilizing mechanism for coexistence (Chesson 2000, Marquard et al. 2013).

We questioned the generality of biodiversity effects, beyond experimental grassland, is there a biodiversity–ecosystem functioning relationship in other systems? Much work has gone into establishing experimental forests with richness gradients, but the growth rate of forest species determines that experimental results will be slow to come (Hector et al. 2011, Morin et al. 2011). However, examination of the relationship using data from natural forest systems is available (Gamfeldt et al. 2013). From an enormous Swedish production forest dataset tree species richness was shown to be positively correlated with multiple ecosystem services, including tree biomass and soil carbon storage (Gamfeldt et al. 2013). Similarly, transpiration, litter production and decomposition have all been shown to have increased rates in richer forest communities (Scherer-Lorenzen et al. 1997, Kunert et al. 2012). These examples provide a solid comparison to a different system, supporting the generality of experimental grassland results. The positive effect of biodiversity on more ecosystem services further strengthens the argument for the role biodiversity has in ecosystem functioning. We expanded on this further in chapter 2, demonstrating that almost half of 418 separate measures of 38 ecosystem processes were significantly affected by plant species richness. By grouping these processes into different categories including biogeochemical cycles, we could generalise that overall species richness effects impact measures of the carbon cycle more than the nitrogen cycle and strongly positively impact higher trophic levels. By comparing many measures of ecosystem processes from a single experimental platform to recent meta-analyses and syntheses that bring together results from many sites, we find further support for the generality of the

importance that biodiversity has on ecosystem functioning Balvanera et al. 2006; Cardinale et al. 2006; Schmid et al. 2009; Hooper et al. 2012).

In the early stages of the Jena experiment, 4 and 6 years after its establishment, we also examined how bryophyte communities had assembled along our species richness gradient. There are few previous studies that consider bryophyte interactions in temperate grassland systems, predictions are difficult as their physiological differences limit how much theory can be borrowed from vascular plant ecology (Pharo et al 1999, Mulder et al. 2001, Müller et al. 2012). Bryophyte richness decreased along the vascular plant biodiversity gradient, predominantly as a result of increasing plant cover. Similar patterns of decreasing bryophyte diversity with increasing productivity have been identified in semi-natural grassland systems (Müller et al. 2012). Changes in bryophyte composition and increasing variation in bryophyte diversity with increasing vascular species richness suggest a role for biodiversity in structuring bryophyte habitat. Advocating one taxonomic group over another for conservation values is premature. In our system we found summed vascular plant and bryophyte community richness was highest when vascular richness was highest, but this may reflect that bryophyte communities were only beginning to assemble.

Community assembly and coexistence

It is difficult to separate pattern from mechanism hence in this section I combine community assembly and coexistence. Our experimental approach throughout this thesis has been to perturb established experimental plant communities and measure their response (chapters 5-10). And for the most part the disturbance we forced on communities was seed addition. Our expectation based on classic theory was that communities are structured by niches (Volterra 1926, Lotka 1932, MacArthur 1972). Niche structuring meant to us that that competitive interactions between species occurred within the confines of the limitations of these niches (MacArthur 1972). By measuring immigration and establishment of the species at successive life history stages we identified opposing patterns during assembly (chapter 5). Seedlings, for all three functional groups (forbs, grasses, legumes) were more abundant when added to resident communities containing the same functional group, when residents were grown

in monoculture. It is possible that this apparent facilitative result could be the result of phylogenetic conservatism of regeneration niches, specifically, plant families potentially share an inherited niche requirement during germination, but this requires much more exploration elsewhere.

Once the species that had been added into communities had established, their productivity was either the same or better when they were added to plots containing different resident species (chapter 5). The productivity response supported our understanding of competition and resource use by different groups of species. We expected that functional groups would assemble in a complementary fashion (Gause 1934, MacArthur and Levins 1967, Pianka 1974, Tilman 1999, Hector et al. 2005, Spehn et al. 2005, Turnbull et al. 2005). A complementary fashion in terms of resource-based assembly rules would dictate that if an immigrant's resource niche overlaps too much with that of the resident species then establishment will not succeed. Our productivity result matched that found in some other seed addition experiments (Fargione et al. 2003, Turnbull et al. 2005, Mwangi et al. 2007), although other results were not consistent (Von Holle & Simberloff 2004, Emery 2007, Emery & Gross 2007, von Felten *et al.* 2009). Similarly we found this pattern repeated in the Jena Experiment when we allowed communities to reassemble with or without seed addition (chapter 6). Communities reassembled in a predictable way, where functional groups were missing, they were complemented by the establishment of the missing functional group. A general consequence of this is that communities converged toward similar levels of species richness, high functional richness, evenness, and phylogenetic richness, as is shown in chapters 9 and 10,. Such convergence has been reported elsewhere and we had come to expect it (Pfisterer et al. 2004, Fukami et al. 2005, Rixen et al. 2008). Again in the Jena Experiment, and under a mildly different scenario, we removed a diversity maintenance regime and opened plots to spontaneous immigration from the local species pool (chapter 8). The impact of the new immigrants on the abundance and stability of communities was lessened in communities with initially higher species richness. These convergence and stability results are expected given we previously maintained these communities at artificially low or high species richness levels (Leps

2003, Diaz et al. 2004, Roscher et al. 2004). However, the patterns of reassembly are of great interest, as they provide far more insight into potential coexistence mechanisms.

The problem with complementary assembly of functional groups as a result is that it is difficult to explain with any hard evidence beyond inferring a mechanism. While it is fine to wholeheartedly believe in niches without ever seeing them, or measuring them directly (Turnbull 2014). It would be far more preferable to quantify their existence. Attempts to directly measure niche complementarity have been largely fruitless (McKane et al. 1990, von Felten et al. 2009), and this lack of results pushed us as with other grassland ecologists to explore other niches beyond resource niches, wherein we turned to coral reefs and tropical forests for inspiration (Janzen 1970, Connell 1971, Connell 1978).

Complementary assembly of functional groups without quantified evidence for resource complementarity begs the question of what other meaningful ecological theme separates functional groups? Functional groups do have a taxonomic basis, generally in grassland experiments grasses (Poaceae – Gramineae) and legumes (Fabaceae – Leguminosae) are considered two functional groups, and all other species are grouped together as forbs (Roscher et al. 2004, Wacker et al. 2009). Although much variation has been found, there is evidence to suggest that closely related species share pests and pathogens (Vandenkoornhuyse et al. 2003, Weiblen et al. 2006, Agrawal 2007, Gilbert and Webb 2007, Fontaine 2009, Futuyma & Agrawal 2009, Gossner et al. 2009). The predominance of resource-niche theory overshadowed what Janzen (1970) and Connell (1971) had proposed from observation of tropical forest tree species, that host-specific predators and herbivores could locally reduce recruitment success of conspecific seedlings. The idea languished as the effect had only been demonstrated weakly, in isolation of a plant community, in forest ecosystems (Augspurger and Kelly 1984, Condit et al. 1992, Packer and Clay 2000, HilleRisLambers et al. 2002, Gilbert 2005, Bell et al. 2006, Freckleton and Lewis 2006). We set about testing whether the effect of reduced immigrant success when grown on soils trained by the same functional group (home effects) versus another functional group (away effects) could be explained by what are essentially Janzen-Connell effects, and we found the accumulation of functional group host specific soil pathogens were responsible (chapter 4). We found

support for this result in previous research demonstrating that species in grasslands do negatively impact conspecific species via the soil compartment, although the agents of the effect were largely mixed or unknown (van der Putten et al. 1993, Bever 1994, Olff et al. 2000, Bever 2003, De Deyn et al. 2003, Bonanomi et al. 2005). In chapter 6 we took this a little further by demonstrating the effect sizes we had found were large enough to regulate coexistence by providing a density-dependent stabilizing mechanism (Chesson 2000).

Evolutionary imprint and trait dispersion

The advent of readily available phylogenetic information and the software for analysis of phylogenetic signal has reinvigorated the search for both assembly rules and the role of biodiversity in ecosystem functions (Kraft et al. 2009, Kembel 2009). In chapters 9 and 10 we found the correlation between species co-occurrence and phylogenetic distance developed in subplots of the Jena Experiment over time. In both analyses phylogenetic overdispersion emerged with time. Phylogenetic overdispersion equates to species in a community being distantly related, conversely, phylogenetic clustering equates to species in a community being closely related (Prinzing et al. 2001, Ackerly 2003, Losos 2008, Cahill et al. 2008, Proches et al. 2008, Vamosi et al. 2009, Thuiller et al. 2010). It is too coarse to proclaim that phylogenetic clustering is caused by the impact of abiotic filters on species with phylogenetically conserved traits (niche conservatism). And likewise invoking limiting similarity as a mechanism for phylogenetic overdispersion is also too simple. Limiting similarity would only cause community composition to become overdispersed if the character of the niche – be it pathogens or a certain functional trait relating to the capture of a specific resource – were consistently phylogenetically conserved. However, phylogenetic conservatism of traits is not universal or consistent (Blomberg et al. 2003, Cavender-Bares et al. 2009, Losos 2011, Pavoine et al. 2013). And modelling of phylogenetic diversity indicators (when trait conservatism has been assumed to be high) also suggests that such indicators have a poor ability to detect niche-based assembly processes (Mason & Pavoine 2013).

In chapter 10 we more closely examined the relationships between co-occurrence and phylogenetic relatedness and found that both very closely and distantly

related species co-occur more over time. This prompted us to examine if patterns of co-occurrence were different for different lineages, which they were, suggesting different levels of phylogenetic dispersion (possibly of traits) within a lineage could be driving interactions of closely related species in different directions. The inclusion of trait information along with phylogenetic information strengthens the case for a role for relatedness. In chapter 11 we demonstrated that coexistence between natives and distantly related aliens in recipient communities of low phylogenetic dispersion could reflect patterns of trait assembly. In communities without aliens, low phylogenetic dispersion corresponds to increased dispersion of most traits, and establishment of aliens corresponds to increased trait concentration. As interesting as this is, it does not bring us much closer to knowing the niche, and hence the mechanism, by which assembly and coexistence in these systems operates (Adler et al. 2013). And it further supports the recent suggestion that we need to return our focus to collecting relevant functional trait data in order to understand the mechanisms that really control community assembly, as laborious as this can be (Mason & Pavoine 2013). In chapter 12 we did just this. Using an index of functional redundancy that we proposed, which is a metric of trait similarity. We found redundancy to increase with species richness, which essentially generates more questions about niche structure. This either suggests that communities have a relatively constant number of niches, but if the abiotic filter permits, more of those niches can be packed by species, or in some habitats there are more niches, and therefore more species can co-exist. We reported trait redundancy as a mean of the traits we collected, however investigating redundancy in a wide variety of single traits in a system could go some distance in demonstrating which traits are critical for coexistence in a specific system, but this is work for another day.

Summary on coexistence mechanisms and community assembly

The results presented here demonstrate the need for a niche (Levine & HilleRisLambers 2009, Turnbull 2014). The niche might be resource based or it might be controlled by pathogens, it is probably both. The critical factor about this niche is that it regulates species through density dependence and this is how it stabilizes core elements of community structure (Chesson 2000, Marquard 2013). These core elements are present

above the species level, and control is likely dictated by traits as opposed to relatedness.

Final word

For someone with a strong interest in community ecology our most fascinating results have been outlined immediately above. However, their broader applicability in a changing world warrants mention. As I noted in the introduction biodiversity loss is undeniable, ongoing and quietly terrifying (Vitousek et al. 1997, Chapin et al. 2000, Sanderson et al. 2002, Balvanera et al. 2006, Secretariat of the Convention on Biological Diversity 2010). Here we have demonstrated the species richness impacts multiple ecosystem functions and that these results are not limited to our experimental system (Naeem et al. 1994, Tilman & Downing 1994, Tilman et al. 1997, Hector et al. 1999, Loreau et al. 2001, Hector & Bagchi 2007, Scherer-Lorenzen et al. 2007, Kunert et al. 2012, Allan et al 2013, Gamfeldt et al. 2013). As we slowly we pick apart how plant communities assemble and coexist, and how coexistence mechanisms maintain species richness, with increasing frequency we end up at the simple conclusion – that biodiversity is critically important.

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Acknowledgements

Many thanks to my lead supervisor Prof. Dr. Bernhard Schmid for his support with experimental design, statistical analysis and scientific writing. I especially want to thank Bernhard for his patience and for not giving up on me.

I wish to equally thank three of my co-supervisors for their support: Christiane Roscher; Andreas Prinzing and Lindsay Turnbull. Christiane, Andreas and Lindsay each supported me in a specific research area, providing guidance with design, statistical analysis, ecological-logic and scientific writing.

I would like to thank Jana Petermann for assistance with data collection, and for the analysis and writing of those chapters on which she has lead. I thank Eric Allan and Tania Jenkins for fruitful discussion on evolutionary ideas, and for their work with the phylogeny and the analysis of phylogenetic signal in the Jena Experiment data. I thank Chris Philipson for his support with statistics and life in general. Similarly I thank (the spectacular late night efforts of) Josephine Haase, Alexa Caduff, and Simone von Burg Black for generally supporting me while we studied together, but also for translating sections of this thesis into German. I am indebted to Jake Alexander for his input into the logic and style of components of this thesis, and for his support in all aspects of life and his general enthusiasm for ecology.

Isabel Schöchli has been a very welcome, continual source of support at what was the Insitute of Environmental Sciences. More recently, Lilian Dutoit has also been very willing to support me get this thing finished.

Alexa Caduff, Daniel Trujillo, Gabriele Cozzi, Isa Kunz, Joel Fiddes and Mikey O`Brien have all made up a most fantastic support network.

I thank Luca Wacker, Susan Eichenberger and Oksana Baudois for field support at our Reckenholz site. I thank Marlen Gubsch, Annett Lipowsky, Elisabeth Marquard and Alexandra Weigelt and for field support at the Jena Experiment, particular thanks to Alex for managing the whole system so adeptly. I thank all of the gardeners involved in the Jena Experiment, especially Gerlinde Kratsch, and the large teams of student who helped Jana and I painfully sort hundreds of kilograms of biomass into species.

I thank Prof. Bastow Wilson for his ongoing support, and for continuing in his own offbeat way to make plant sciences pretty fantastic.

I would like to generally thank all staff associated with the following institutes/departments/experimental platforms: Max Planck Institute for Biogeochemistry in Jena; The Jena Experiment, Agroscope FAL Reckenholz in Zurich; what was the Institute of Umweltwissenschaften at the University of Zurich, what is now the Institute of Evolutionary Biology and Environmental Studies at the University of Zurich; Unité Mixte de Recherche Ecobio: Ecosystèmes - Biodiversité – Evolution, Université de Rennes.

I must thank my parents Jon and Lynley who supported me always during this endeavour, I have dedicated this thesis to Ma, who never got to see it finished, but who would never have forgiven me if I had not completed it. Thanks also Morgan and Jessie for further encouragement towards the end.

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